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SOIL SCIENCE

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A STUDY OF METHODS FOR THE DETERMINATION OF THE AVAILABLE POTASSIUM OF SOILS[1]

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Wisconsin Agricultural Experiment Station

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Of the large amount of potassium which generally exists in soils, a small portion is usually much more available than the remainder. Plants undoubtedly draw largely on the more available portion for their needs. Because of the great variability in origin and subsequent cropping and treatment of soils there is a large difference in the amount of available potassium in different soils. It was the purpose of this investigation to study the methods now in use for measuring the supply of available potassium in soils and to make any improvements possible in the methods. The probability that the potassium existing in the exchange complex of the soil largely makes up the readily available portion is suggested by the work of Gedroiz (11) who estimated that only 6 per cent, on an average, of the total potassium is in exchangeable or replaceable form. Methods for determining the available potassium should, therefore, concern themselves largely with evaluating this form, especially if it can be shown that plants are largely dependent on this form to supply their needs.

REVIEW OF LITERATURE

Investigators have suggested numerous solvents and methods for extracting the available portion of the mineral elements of soils. Gerlach (12) and Schloesing (28) proposed the use of carbonated water as a solvent. Hofmeister (16) tried humic acid as a solvent. Maxwell (22), in his studies of Hawaiian soils, employed 1 per cent aspartic acid and recommended its use. Dumont (4) speculated upon the forms of potassium in soils but did not report any analytical work.

It was in 1894 that Dyer (5) suggested the use of one per cent citric acid as a solvent to extract available potassium. Working with Rothamsted soils which had received long continued fertilizer treatments, he attempted to correlate the amounts of essential elements extracted by citric acid with the actual yields of crops. This work was later further supplemented and verified by additional studies (6), the results of which appeared in 1901. He found the yields of barley to be closely correlated with the amounts of citric acid-soluble potassium. Dyer (6) decided that if less than 10 mgm. of potassium are dissolved from 100 gm. of soil, potassium fertilization is necessary. An interesting observation was made by

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[1] Abstract of a thesis submitted to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of doctor of philosophy. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

[2] For the many suggestions and helpful criticisms received, the author desires to thank Prof. Emil Truog under whose general direction the work was done.

Dyer to the effect that when magnesium or sodium salts were added in the fertilizer treatment, potassium additions remained more soluble.

Hall and Plymen (15) in 1902 reported the results of some investigations of the Broadbalk field at Rothamsted which had grown wheat for 50 years. Using 1 per cent citric acid, 1 per cent acetic acid, and 0.04 normal carbonic acid, they found that the most favorable comparisons between productivity and soluble potassium were obtained with the citric acid. Hall and Amos (14) tried the effect of repeated extractions with these solvents and were forced to employ citric acid only, because of the deflocculation brought about by the other two solvents. They concluded that for ordinary analytical purposes, the single extraction is likely to give as much information as repeated attacks of the same solvent. Wood (31) found that 1 per cent citric acid was effective in differentiating several barley soils, and that those soils that responded strongly to potash fertilizers yielded an average of 7.3 mgm. of soluble potassium per 100 gm. of soil, whereas those that did not respond yielded on an average 14 mgm.

Since 1920, German investigators have experimented with several solvents and have checked the results with field experiments. König, Hasenbäumer, and Schafers (18) in 1923 studied the mineral requirements of the potato and compared them with the available supply in the soil as determined by steaming the soils and by extraction with 1 per cent citric acid. These investigators were able to get good correlation between the amounts of potassium removed by the potato crop and the amounts removed by steaming for a 5-hour period. When 8 mgm. or more of soluble potash (K_2O) were removed, by steaming, from 100 gm. of soil, potash fertilization was unnecessary. There seemed to be definite proof that if the steam-soluble potash was less than 5 mgm. in 100 gm. of soil, fertilization was required. If the amount extracted ranged between 5 and 8 mgm., there was some uncertainty as to the requirement. The same general results were obtained with citric acid, although this solvent dissolved larger amounts than steam.

König, Hasenbäumer, and Kroger (19) carried on similar studies with oats and found that the oat crop extracted 93.5 to 105.4 per cent as much potassium as the steam extraction. Thus, again it appears that the amount dissolved by the steam treatment just about equals the amount that is available for crop use. Citric acid, however, as used by these investigators, extracted about five times as much as the plants were able to utilize. These authors quote the results that Liebscher obtained by using a 10 per cent solution of hydrochloric acid. With this solvent, Liebscher found that if 500 mgm. of potash (K_2O) can be dissolved from 100 gm. of soil, no potash fertilization is necessary, but if only 150 mgm. are soluble, a definite potash requirement is indicated. König and Hasenbäumer (17) also concluded that steaming a soil for five hours at five atmospheres pressure gives a good indication of the amount of replaceable potassium in the soil. They noted a relationship between the total potassium of the soil and that soluble in both steam and citric acid. A suggestion of Lemmermann regarding the solution of material by successive filtrations was tried, but because of deflocculation effects with nearly all of the soils, the method was found to be too slow for ordinary use. They also tried successive extractions, but concluded that several extractions gave no better indications than one. Treatment of soils with 10 per cent ammonium chloride solution was found to be especially useful in determining the amount of readily replaceable potassium. The amounts extracted by this treatment always exceeded those obtained with citric acid or with steam. König and Hasenbäumer concluded that if less than 16 mgm. of K_2O is dissolved from 100 gm. of soil with 1 per cent citric acid, the soil needs a potassium fertilizer.

The most original of the newer methods of attacking the problem is the one devised by Neubauer (23, 24, 25, 26) in 1923. His method is a physiological one which aims to determine the amount of available soil nutrients by determining the amount that a comparatively large number of rye seedlings will extract when grown for 18 days on a small mass of the soil. The assumption is that the young plants have a strong feeding power for nutrients during the early days of their existence. The ability of the soil to supply them at a rapid rate is thereby determined. After being ashed, the plants are analyzed by the usual analytical procedures. Certain minimum requirements are set for each crop and if the soil does not yield these amounts, fer-

tilization is advised. This new method provided the stimulus for considerable work, especially by Engels and Hirschberger (8), by Roemer (27), by Densch and Pfaff (3), and by Gunther (13). The first two workers found the method applicable to and indicative of the field conditions. In the case of several soils yielding less than 18 mgm. of K_2O by the Neubauer test, responses to potassium fertilizers were obtained in the field. Soil from plots which had not been fertilized with potassium yielded 25.2 mgm. to Neubauer tests, whereas soil from two plots which had received potash fertilizers for 10 years yielded 46.4 and 49.8 mgm., respectively.

Roemer found it necessary to revise Neubauer's minimum requirements in order to meet the soil conditions at Halle, but considered the method a useful one. Densch and Pfaff were not so well satisfied with the method. Gunther investigated the effect of intensity of light and the reaction of the soil on the amount of nutrients taken up by the plants. Light from a northern exposure caused an assimilation of 5 to 6 mgm. more than any other exposure. In the range of pH from 5 to 8 no differences in plant activity were observed. Egner (7) in his work with several Swedish soils found consistent correlations between the Neubauer test and field experiments. Neubauer estimates that 85 per cent of the recommendations based on the test have been correct.

McGeorge (20,21) in working with Hawaiian soils used several solvents, including 1 per cent citric acid and 0.2 normal nitric acid. He concluded that the citric acid-soluble potassium gave the best indications of the probable need for potassium fertilizers in the field. The quantity of water-soluble potassium in Hawaiian soils is small, and McGeorge believes it comes largely from the hydrolysis of minerals. The presence of absorptive gels in soils is believed by him to be a disturbing factor in making water extractions. McGeorge found that acid soils were uniformly low in soluble potassium. He did not find a relationship between the amounts of soluble silica and potassium such as would exist if potassium silicates dissolved as such. A hydrolysis reaction is thus indicated. Using 1 per cent citric acid, he found that soils giving no response to fertilization yielded on an average 55 mgm. of soluble K_2O per 100 gm. of soil, whereas soils giving responses to fertilization yielded on an average 14 mgm.

Fischer (9) pointed out the importance of the presence of other cations, especially calcium, upon potassium availability and its use by plants. This relationship may be important in making comparisons of the potassium that plants can absorb from different soils. The utilization of plants as indicators of potassium deficiency in soils is no doubt influenced by these considerations.

EXPERIMENTAL

Judging from a review of the literature, there appear to be several possible chemical methods of ascertaining the fertilizer needs of a soil. The interpretation of the results obtained by each method should undoubtedly be made in accordance with soil and climatic conditions in the different localities. Consequently, it seemed advisable to make a comparative study of these methods upon a group of soils with the idea of finding out, if possible, which methods merit continued use. Moreover, it was hoped that new means of attack might be developed. With these purposes in mind, the following lines of inquiry were attempted with 10 samples of soil:

1. Preliminary examination of the soil samples
   a. Mechanical composition
   b. Colloidal content
   c. Lime requirement
   d. Volatile material

2. Neubauer tests
3. Neubauer tests with fertilizer additions
4. Availability studies by single extraction with different solvents
   a. Distilled water
   b. Citric acid (1 per cent)
   c. Carbonic acid (0.05 normal)
   d. Ammonium chloride solution (10 per cent)
   e. Steam
   f. Steam with citric acid (1.0 per cent)
5. Availability studies by successive extractions with normal ammonium chloride

Materials and methods

Soils. The soils used in the investigation were collected from widely separated localities in Wisconsin. They were passed through a coarse screen to remove stones, stubble, and other debris, mixed thoroughly, and then stored in a cold room in a slightly moist condition. Samples taken for analysis were again screened through a 2-mm. screen, air-dried, and stored in air-tight Mason jars. Samples for Neubauer tests were also screened and placed in Mason jars, and then stored in a cold room. Texturally, the soils varied from loamy sand to silt loam.

Physical analyses. The mechanical composition of the soils was determined according to the regular procedure of the U. S. Bureau of Soils. Percentages of colloidal matter were determined by the hydrometer method of Bouyoucos (1). The amount of volatile matter was found by igniting 10-gm. samples at red heat for 30 minutes.

Neubauer tests. The Neubauer tests were made as follows: To a sample of soil of 100 gm., there was added and thoroughly mixed 50 gm. of washed quartz sand. The mixture was then spread evenly over the bottom of a flat dish having a diameter of about 11 cm. and a height of 7 cm. Two hundred and fifty grams of quartz sand was then moistened slightly with distilled water, and about one-half of this was spread over the soil-sand mixture in the dish, and at the same time a short glass tube was inserted near the center of the dish with its lower end resting on the soil-sand layer. This was used later for adding water to the lower layer. One hundred rye seeds which had previously been treated with weak formalin solution were then distributed evenly over the surface of the sand. The remainder of the moist quartz sand was then placed on top and uniformly packed over the seeds. The moisture content of the whole soil mass was regulated by weighing. Neubauer suggests the use of 80 gm. of water in all cases, but it was found necessary to reduce this to about 65 gm. for some of the more sandy soils. Duplicate tests were made in all cases. Covers were then put on the vessels and the entire group was set on a table in front of a north window of the laboratory. In about 48 hours the shoots began to appear, and in 96 hours the covers were removed. Henceforth it was necessary to keep the containers up to a definite weight by adding distilled water daily. After 18 days the plants were removed from the dishes and the roots washed free of soil with running water. A screen was used to catch all of the fine roots,

loose seed coats, and leaves. After being allowed to dry for a few hours, the material was cut into convenient lengths, placed in porcelain crucibles, and ashed in an electric muffle furnace. If the roots and seeds are placed in the bottom of the dish and covered with the leaves, there is no danger of loss by sudden popping and snapping of the old seeds. The ash was extracted by heating with water for two hours on a hot plate. After filtration and washing, the determination of potassium was made in the filtrate according to the usual platinic chloride method. The use of acid in dissolving the potassium from the ash seems to be unnecessary. By conducting a Neubauer test on quartz sand alone a value was obtained for the potassium in the seed and that extracted from the quartz sand. This value was subtracted from the amount found in the plants grown on a soil. The difference was assumed to have been taken from the soil.

Neubauer believed potash fertilization to be unnecessary if rye and wheat are able to extract 14 mgm. or more of K_2O from a soil; if they extract less than 14 mgm. he believes potash fertilization to be necessary, and the greater the discrepancy, the greater is the need of potash. Similarly other crops are given minimum potash requirements by him as follows: barley and oats, 18 mgm.; red clover, 29 mgm.; alfalfa, 34 mgm.; potatoes, 37 mgm.; sugar beets, 39 mgm. Inasmuch as rye was used in this work, the value of 14 mgm. was taken as a standard in deciding relative potassium deficiencies of the soils.

Chemical analyses. Lime requirements were determined by the Truog method (29). The reactions of the water extracts were determined colorimetrically according to the general methods outlined by Clark (2). The ratio of 1 part of soil to 10 parts of solvent was employed in the extractions of the untreated soils to determine the amount of available potassium in them. These solvents stood in contact with the soils about 24 hours, during which time two shakings, each of 1 hour duration, were given the samples in an end over end rotary shaker. Filtration was accomplished by the use of a Büchner funnel and a special suction bulb designed and described by Truog (30). In this method the soil forms an ultra-filter, and an exceptionally clear solution is obtained. Analyses were made on aliquot parts of the extracts without allowing them to stand in contact with the glass containers for more than 48 hours.

Potassium was determined in two ways; namely, by the usual platinic chloride method and by a new test[3] devised by Truog. In this new test, advantage is taken of the distinctive violet flame which develops when a potassium salt is volatilized. By means of special apparatus the intensity of the violet flame produced by the unknown test solution is compared with a standard and in this way the amount of potassium present is determined. The test is excellent for soil extracts because it works well with low concentrations. It saves much time and labor.

Fusions for total potassium were made by the method of J. Lawrence Smith.

[3] To be described elsewhere.

In the steam extractions, a pressure cooker was employed in which a pressure of 15 pounds above atmospheric pressure was developed. By placing the soil in an Erlenmeyer flask together with just enough solution to saturate it, the steam was allowed to attack the particles directly. After steaming for 4 hours

TABLE 1

The mechanical composition of the soils

SOIL		PERCENTAGES OF THE SEPARATES (AVERAGES OF DUPLICATES)						
Number	Class	Fine gravel	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt	Clay
1	Sandy loam	0.150	2.488	24.620	31.810	11.910	20.702	8.320
2	Sandy loam	1.400	12.780	24.910	30.930	7.385	17.645	4.950
3	Loamy sand	2.100	15.890	30.460	31.690	4.680	10.423	4.757
4	Sandy loam	1.125	7.510	18.450	31.010	7.270	25.860	8.775
5	Silt loam	0.500	1.870	2.200	12.450	9.150	67.030	6.800
6	Sandy loam	2.560	8.850	25.530	21.730	11.780	23.515	6.035
7	Sandy loam	2.810	10.570	26.920	23.515	10.835	19.940	5.410
8	Sandy loam	2.100	12.260	25.045	16.460	11.730	25.770	6.635
9	Silt loam	0.295	0.890	1.226	12.265	9.020	66.780	9.524
10	Silt loam	0.790	2.275	3.210	9.480	15.950	60.400	7.895
11	Muck	Not analyzed, being a muck						

TABLE 2

Predominating minerals in the fine sand separates

SOIL		OBSERVATIONS
Number	Class	
1	Sandy loam	High content of quartz—very little feldspar
2	Sandy loam	Medium quartz content—considerable feldspar
3	Loamy sand	General mixture of many minerals—little feldspar
4	Sandy loam	Similar to number 3
5	Silt loam	Many minerals, especially feldspars and mica
6	Sandy loam	Similar to number 3 with more mica present
7	Sandy loam	Resembles number 6
8	Sandy loam	Resembles number 6
9	Silt loam	High content of quartz, many colloidal aggregates
10	Silt loam	General mixture of many minerals, highly colored
11	Muck	Muck. Mineral matter slightly visible

the solutions were made up to a weight of 10 times that of the soil, and the flasks were shaken as for other extracts.

Preliminary examination of the soils

In order more fully to understand the nature of the soils used in this investigation, it seemed essential to determine their textural characteristics, content of colloidal material, lime requirement, and content of volatile matter. Micro-

scopical examinations of the fine sand separates were also made to gain some insight into the mineral composition of each soil.

In the group of 11 soils selected, there were 3 silt loams, 1 loamy sand, 6 sandy loams, and 1 muck. In tables 1, 2, and 3 the mechanical composition,

TABLE 3

Volatile matter, colloidal material, and lime requirement of the soils

SOIL		LOSS ON IGNITION	COLLOIDAL MATERIAL	LIME REQUIREMENT
Number	Class			
		per cent	*per cent*	*pounds per acre*
1	Sandy loam	2.87	19.5	6,000
2	Sandy loam	3.46	13.5	4,000
3	Loamy sand	1.91	11.0	6,000
4	Sandy loam	6.78	21.0	6,000
5	Silt loam	5.91	33.0	4,000
6	Sandy loam	3.29	16.5	6,000
7	Sandy loam	3.92	14.5	4,000
8	Sandy loam	4.59	17.5	6,000
9	Silt loam	5.31	34.5	4,000
10	Silt loam	6.08	32.5	6,000
11	Muck	37.62	20.0	None

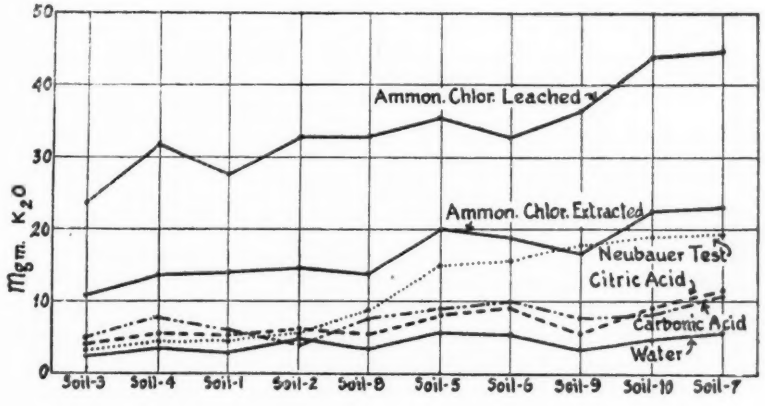

FIG. 1. POTASH REMOVED FROM 100-GM. SAMPLES OF SOILS BY METHODS INDICATED

the predominating minerals, the colloid content, the loss on ignition, and the lime requirement are given.

Results of Neubauer tests

The results of the Neubauer tests are presented in table 4. The data are also represented graphically in figure 1.

From the data, it is evident that, according to the test, four of the soils are not in need of potassium additions, one is on the border line, and the other six should respond to fertilizer. It is clear that the results obtained in this test with soil number 7 are consistent with those obtained from chemical tests. According to the plant tests there was a slight deficiency of available potassium in the muck soil, yet all of the solvents with the exception of carbonic acid showed an abundance of the soluble form. In this soil the potassium probably showed a rapid initial solubility, giving rise to a strong concentration at the beginning, which is not easily maintained during the growing period of the seedlings. In contrast to this soil, number 7 was able to build up a strong initial concentration and maintain it during the entire feeding period of the plants.

TABLE 4

Available potash in soils as determined by the Neubauer test

SOIL NUMBER	TOTAL POTASH IN ASH	POTASH IN ASH IN CHECK	POTASH TAKEN FROM SOIL	MINIMUM POTASH REQUIRED	DEFICIENCY
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	16.72	11.90	4.62	14.00	9.38
2	17.67	11.90	5.77	14.00	8.23
3	14.02	11.90	3.12	14.00	10.88
4	16.35	11.90	4.45	14.00	9.55
5	26.35	11.90	14.45	14.00	None
6	27.26	11.90	15.36	14.00	None
7	31.68	11.90	19.78	14.00	None
8	20.35	11.90	8.45	14.00	5.55
9	29.18	11.90	17.28	14.00	None
10	30.14	11.90	18.24	14.00	None
11	23.81	11.90	11.91	14.00	2.09

Soil number 10 gave up the second largest amount of potassium to the seedlings. In the solubility studies it was consistently high in soluble potassium, except where water was the solvent. Soil number 9 ranked low in available potassium as measured by most of the solvents; yet, it furnished the third largest amount to the seedlings. Again, numbers 5 and 6, though high in the order of availability with solvents, were just over the minimum value in the Neubauer tests. Of the six soils below the Neubauer minimum, soil number 3 was the lowest. Studies with solvents, repeatedly placed this soil lowest in available potash. The soil was consistent throughout. The other five soils in this group, with the exception of number 2, were also relatively weak in furnishing potassium to either solvents or seedlings. Soil number 2 perhaps resembled number 11 in that it appeared to contain an appreciable amount of readily soluble potassium, but when plants began to remove it, the soil could not maintain the supply. Thus, in a general way the Neubauer experiments

agree well with the results obtained with solvents, and especially with those obtained by the use of citric acid, steam, and ammonium chloride.

Results with modified Neubauer tests to include additions of potassium

In the wire basket method used by Gardner (10), only a few seedlings were grown on a limited mass of soil. Because of the limited areas into which the roots permeated, there was great difficulty in obtaining concordant results. The possibility of making rapid determinations of plant responses to fertilizers by the use of the Neubauer arrangement was the object of three preliminary experiments. The three soils chosen for study were number 7, which yielded the highest amount of potassium to the seedlings in the regular test; number 11, which was rich in potassium according to various solvents, yet did not meet the Neubauer requirements; and number 3, the poorest of the entire

TABLE 5

Weights of air-dry roots and tops of rye grown in Neubauer tests modified to include fertilizer treatments

TREATMENT	WEIGHTS OF AIR-DRY ROOTS AND TOPS OF RYE		
	Soil No. 7	Soil No. 3	Soil No. 11
	gm.	*gm.*	*gm.*
No treatment	3.42	2.05	2.52
KCl alone—500 pounds per acre	3.59	3.55	3.39
$CaH_4(PO_4)_2$—500 pounds per acre	3.40	2.30	2.84
$CaH_4(PO_4)_2$ and KCl	3.68	3.82	4.10

group. Solutions of potassium chloride and mono-calcium phosphate were prepared so that 10 cc. to a jar were equivalent to an application of 500 pounds an acre of surface soil (2,000,000 pounds). The experiments were set up in exactly the same way as for the Neubauer test, and in addition potassium alone and in combination with other fertilizers was added to the soil in certain jars. Thus, there were two jars of unfertilized soil, two fertilized with potassium, two with phosphorus, and two with a combination of phosphorus and potassium. The plants were allowed to grow 14 days. The photographs taken at the end of this period are shown in plate 1. The weights of air-dry material for the series of three soils are given in table 5.

The photographs and weights show that, in the case of soil number 7, an addition of 500 pounds of potassium chloride gave no additional growth over that in the check. An addition of phosphorus alone similarly showed no effect, and the combination of potassium and phosphorus likewise was ineffective. In contrast to this, both the photograph and the weights of plant material show that number 3 did respond to a potassium addition, either alone or in conjunction with phosphorus, but phosphorus alone was of little value during

this short growth period. The responses of soil number 11, as shown in the table, were similar to those for number 3, the effects being, however, somewhat less marked.

The results of this study seem to warrant the use of the modified Neubauer test as a rapid means for obtaining information regarding the responses which may be expected from potassium fertilization. In these studies, limited as they are, there is a perfect correlation of the results with those obtained by other more laborious means.

Extraction of potassium with different solvents

The effect of the different solvents on potassium in soils, together with the total potassium in each soil, is given in table 6. The data are also represented graphically in figure 1.

TABLE 6

Potash, total and available, in the soils

SOIL NUMBER	TOTAL K_2O	AVAILABLE K_2O BY DIFFERENT EXTRACTANTS IN 100 GM. OF SOIL					
		Distilled water	1/20 normal H_2CO_3	One per cent citric acid	Ten per cent NH_4Cl	Steam	One per cent citric acid and steam
	per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	1.76	2.92	6.14	5.88	13.76	3.05	6.00
2	1.50	5.45	4.83	6.07	14.68	4.10	6.00
3	1.65	2.78	4.60	4.49	10.98	3.00	5.20
4	1.96	4.48	7.50	5.88	13.32	5.00	6.30
5	2.28	5.51	8.50	8.17	19.98	5.80	8.50
6	1.63	5.08	9.66	9.37	18.36	4.50	7.70
7	1.74	5.80	10.63	11.55	22.71	6.70	9.20
8	1.72	3.38	7.34	5.80	13.52	3.50	5.50
9	2.01	3.38	7.34	5.40	16.91	4.00	6.20
10	2.09	4.60	8.21	8.70	22.48	5.50	7.80
11	1.12	6.46	7.64	11.46	27.21	7.00	10.40

It will be noticed that soil number 5 was highest in total potassium, followed by numbers 9 and 10. All three soils are silt loams. Among the sandy loam soils, number 4, a prairie soil, was the richest in potassium. Number 1 was next in order, followed by numbers 7, 8, 6, and 2. Soil number 3 is a loamy sand, and contains more potassium than number 2, which is a sandy loam. Number 11, the muck soil, was lowest in potassium. These quantities of potassium are quite consistent with the amounts usually present in soils of this region.

That there is no relationship between the total amount of potassium in these soils and that readily soluble is strikingly shown by the data. The only case of a possible correlation is with soil number 5. With water and carbonic

acid extraction it was third highest, and with steam or with steam and citric acid extraction it was second highest. With cold citric acid extraction it was fifth, however, and ranked fourth in yielding potassium to the ammonium chloride solution. The muck soil, which was lowest in total potassium, was rich in the readily soluble forms. Soil number 7 contained only an average quantity of total potassium, but was well supplied with the soluble forms. Soil number 3 was also consistent in that it was lowest in soluble potassium with all solvents used. Soils number 1 and 8 were likewise generally low in soluble potassium. Number 9, although high in total potassium, was not able to furnish much potassium to any solvent.

With regard to the action of different solvents upon the same soil, it may be noted that the 10 per cent ammonium chloride treatment removes the largest amount of potassium in all cases. This is made possible, no doubt, because of the vigorous replacement of soil bases which the ammonium radical is capable of accomplishing. Gedroiz used this solution in comparative studies of the soil types of Russia, and its vigorous replacing action is well known. In certain respects, this solution gives results which seem to place these soils in their proper order of productivity as regards potassium. The method of extraction with ammonium chloride results in difficulty, however, in the analytical procedure because the ammonium salts used in large amounts creep over the edges of the containers upon evaporation of the extracts.

Citric acid yields data which correlate fairly well with the Neubauer tests. Although not so vigorous in its action as ammonium chloride, its degree of differentiation and sensitivity is about the same. In addition, the extracts with it are much easier to manipulate analytically. It dissolves slightly more potassium from some soils when used in connection with steam, but there seems to be no advantage in using it in this way. If the work of Dyer and König and Hasenbäumer is used as a basis for interpretation of the data, this entire group of soils would be classed with those requiring potash fertilization. Since two soils, at least, are not responsive to such additions, it seems that the reference figures of these investigators must be lowered if citric acid is to be applied to soils of this region. On the basis of other data presented in this paper it appears that about 10 mgm. or more of potash in 100 gm. of soil should be soluble in 1 per cent citric acid; otherwise, fertilization would be advisable.

Carbonic acid is capable of dissolving as much potassium as is citric acid, but the two acids are quite unlike in their effects. With certain soils, such as numbers 1, 4, 8, and 9, carbonic acid dissolved more than citric acid. These soils are rather high in volatile matter. However, in the case of the muck soil, the carbonic acid was quite inactive and did not give any indication of the high availability of the potassium as shown by other tests. It is possible that the presence of other soil bases capable of forming rather insoluble carbonates was a disturbing factor in the use of this acid. In general, results with carbonic acid do not correlate closely with the known facts regarding the potash needs of this group of soils.

Distilled water gives results which are generally indicative of the amount of potassium available to plants. The only soil which was not correctly evaluated by this solvent was number 10. With other solvents and in pot experiments this soil appeared as one of the best in the group, although it was low in water-soluble potassium. With water extraction, deflocculation often takes place and then filtration is extremely slow. The range in amounts extracted from these soils with water is not a wide one, and hence the differentiation between samples becomes more difficult. Analytical errors in determining these small quantities are also serious. The method is thus not generally satisfactory.

Steam removes more material from soils than does cold water. The data obtained with steam enable one to evaluate the soil correctly with regard to its soluble potassium. The correlation between steam-soluble potash and crop response to potash fertilization is a good one. According to the reference figures of König and Hasenbäumer for this method, the entire group of soils requires fertilization with potash. The method is somewhat more cumbersome than others and requires more time and apparatus. Yet it appears to be a good way of measuring the available potassium.

In general it is evident that with the average soil, each solvent will have specific effects, depending to a large extent upon the nature of the solvent, its concentration, and upon the form in which the soil potassium exists. Consequently the choice of a solvent for such work should be based upon the criterion of plant growth. This may be best accomplished by a comparison with fertilizer results obtained in field or greenhouse. On the basis of this comparison the results with the 1 per cent citric acid appear to be good, those with ammonium chloride are better, and those obtained by steaming are likewise worthy of serious consideration.

On the grounds of these experiments, it appears that if from 100 gm. of soil less than 10 mgm. of potash is soluble in 1 per cent citric acid, or less than 20 mgm. in 10 per cent ammonium chloride solution, or less than 6 mgm. in steam extraction, the soils will respond to an addition of potassium fertilizer.

Available potassium by successive extractions with normal ammonium chloride solution

The ammonium chloride extractions of potassium having correlated with plant indications of available potassium in a rather consistent manner, further studies were made, a normal solution of this salt being used and the soils leached successively with it rather than a single extraction made as was done previously. A sample for analysis consisted of 200 cc. of leachings and a 20-gm. sample of each soil was leached with a total volume of 1 liter. Analyses of the leachings were made in the usual manner. The data obtained by this procedure should be useful in indicating the total amount of the potassium existing in the absorption complex, because by this method the products of the replacement reaction are continually removed and there is no oppor-

tunity for a condition of equilibrium to develop which would limit further solution. The results of these leachings are presented in table 7, in which the data are expressed on the basis of 100-gm. samples of soil.

Comparisons of the total potassium removed by this procedure and that extracted in the Neubauer test show an excellent correlation between the two methods. The quantities, to be sure, are much larger in the case of the extraction by leaching, but the order of availability indicated within this group of soils is nearly identical with the two methods. It appears, therefore, that this method of attack yields information which is indicative of the probable ability of the soil to supply plants with potassium. This should be the criterion for judging the value of any extractant. If the plant activity in extracting potassium can be closely simulated, results of value in judging the fertilizer needs of soils may be accurately determined.

TABLE 7

Replaceable potash in soils displaced by successive leachings with normal ammonium chloride solution

SOIL NUMBER	POTASH REPLACED IN 100 GM. OF SOIL					
	First 1,000 cc. of extract	Second 1,000 cc. of extract	Third 1,000 cc. of extract	Fourth 1,000 cc. of extract	Fifth 1,000 cc. of extract	Total in 5,000 cc. of extract
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	10.0	6.0	5.0	3.5	3.0	27.5
2	11.0	7.5	6.0	4.5	3.5	32.5
3	8.5	6.0	3.5	3.0	2.5	23.5
4	10.5	7.5	5.5	4.5	4.0	32.0
5	11.5	8.5	6.0	5.0	4.5	35.5
6	11.0	7.5	6.0	5.0	3.5	33.0
7	14.5	10.5	8.5	6.0	5.0	44.5
8	11.0	7.5	5.5	4.5	4.0	32.5
9	11.5	8.5	7.0	5.5	4.5	37.0
10	14.0	11.0	8.0	6.0	5.0	44.0

In order to determine the nature of the replacement reaction in soils, the equation for expressing the conditions governing the heterogeneous equilibrium of a solid dissolving in a liquid was applied to these data. This equation states that the rate of solution is proportional to the difference between the original strength of the solvent and the strength at the time of measurement, and may be expressed as:

$$\frac{dx}{dt} = K\,(A - x)$$

The assumption must be made that the surface area of the solid remains constant. The formula was given a different meaning in the present instance, however, by taking as the independent variable, the volume of leachate ob-

tained, which correspondingly changes the value of A, making it equal to the total material dissolved, and x to the amount dissolved in the corresponding volume of leachate. The revised formula may thus be written:

$$\frac{dx}{dv} = K(A - x) \qquad (A)$$

Integrating this expression gives:

$$K = \frac{1}{v} \log_e \frac{A}{A - x} \qquad (B)$$

K may be termed the velocity constant of the solubility reaction. In applying this formula the values presented in table 7 may be utilized, that is, the last column presents values of A, the five different leachings present values of x, and the corresponding total volumes of leachate represent v. The results of this study are presented in table 8.

The values of K (the velocity constants) are interesting in that they show a constant reaction rate for the first three leachings, but after about 3,000 cc. have passed through the soil, the rate of solution slows down. The similarity of the constants for the 10 soils points to the existence of the same potassium-containing compound in the different soils. The replacement of potassium takes place at a constant rate under the influence of leaching until there is but a trace of replaceable potassium left, after which a steady solution of the difficultly soluble potassium minerals takes place at a slower rate. The amount of potassium held in the exchange form therefore can be calculated from the formula, since K is a constant, by determining the value of x and the corresponding volume of leachate. To illustrate the principle of this method for obtaining total replaceable potassium, the following calculations are made:

$$\log_e \frac{A}{A - x} = vK$$

From (B)

Let $v = 200$ cc. of extract and $K = 21.90 \times 10^{-4}$

Then

$$\log_e \frac{A}{A - x} = vK = 0.4380$$

and

$$\frac{A}{A - x} = 1.55$$

and

$$A = \frac{1.55\,x}{0.55}$$

With soil number 3, $x = 8.5$.

Total replaceable potassium, $A = \frac{1.55 \times 8.5}{0.55} = 23.9$ mgm.

By analytical determination $A = 23.5$ mgm.

To test the applicability of this method of determination, all of the soils

TABLE 8

Velocity constants for the solubility of potash in the soils when leached with normal ammonium chloride solution

SOIL NUMBER	VELOCITY CONSTANTS ($K \times 10^{-4}$)			
	First 1,000 cc. of extract	Second 1,000 cc. of extract	Third 1,000 cc. of extract	Fourth 1,000 cc. of extract
1	23.68	22.80	24.07	28.00
2	21.34	21.80	22.80	27.81
3	25.06	24.50	24.85	28.82
4	21.20	21.89	22.90	25.62
5	21.72	21.57	22.46	25.52
6	21.34	21.80	22.80	27.81
7	21.18	21.29	23.22	26.83
8	21.34	21.90	22.83	26.32
9	20.26	20.02	22.28	26.24
10	21.02	20.98	22.74	26.84
Average.....	21.88	21.92	23.10	26.98

TABLE 9

Total replaceable potash in soils as observed by experiment and as calculated by use of the formula

SOIL NUMBER	REPLACEABLE POTASH IN FIRST 1,000 CC. OF LEACHING	TOTAL REPLACEABLE POTASH OBSERVED IN FIVE LEACHINGS	TOTAL REPLACEABLE POTASH CALCULATED
	mgm.	mgm.	mgm.
1	10.0	27.5	30.0
2	11.0	32.5	33.0
3	8.5	23.5	25.5
4	10.5	32.0	31.5
5	11.5	35.5	34.5
6	11.0	33.0	33.0
7	14.5	44.5	43.5
8	11.0	32.5	33.0
9	11.5	37.0	34.5
10	14.0	44.0	42.0

were examined in this manner and the results are presented in table 9. The agreement between the observed and calculated value for total replaceable potassium is good. This formula may be utilized in shortening the time required for a determination of total available potassium. It is necessary only to leach a 20-gm. sample of soil with 200 cc. of normal ammonium chloride solu-

tion and determine its content of potassium. The total replaceable potassium will be three times this quantity, as is evident from a study of the formula.

GENERAL DISCUSSION

The supposition that the potassium used by plants is largely furnished by an exchange complex rather than by primary soil minerals directly seems to be clearly substantiated by many of the data presented in this paper. There is only a small yearly loss of potassium in drainage waters; yet plants are able to extract relatively large amounts in a short time from the soil. This apparently means that there is a reservoir of readily exchangeable and hence available potassium maintained in each soil, and that plants utilize this supply rather than that represented by the difficulty soluble minerals. Consequently, as the potassium-bearing minerals slowly dissolve, the potassium liberated is taken up by the exchange complex of the soil. In this combination the potassium is not readily leached away and yet is quite easily available to plants. Variations in the capacity of different soils to supply potassium to plants are therefore due to variations in the exchange complex of the soils and also to the capacity of the primary potassium minerals to keep the exchange complexes saturated. Determinations of the amount of water-soluble potassium are therefore valuable only in a series of comparative studies on one soil, and extending over considerable periods of time.

Replacement methods involving the use of concentrated salt solutions aim by mass action to saturate a soil with one base, and to throw into solution at the same time an equivalent amount of other bases, which may then be measured by appropriate analysis. Such a procedure shows the relative absorptive capacities of different soils, but since the reactions are of the balanced type with one reactant of unknown nature and concentration, it is probable that a certain strength of replacing solution will not remove a definite proportion of the absorbed bases from all soils. Moreover, great differences exist in the relative quantities of the different bases held by soils and since certain of these are held more strongly than others, there will be wide variations in the relative proportions of the several bases found in an extract. Consequently, in a soil already highly charged with calcium a replacing solution may yield an extract rich in this base and low in potassium. However, this soil may really have a large quantity of available potassium. The strength with which different bases are held in the base exchange complex is indeed important, and potassium is among those most strongly held.

The use of one per cent citric acid as a measure of potassium availability has been quite successful in European laboratories. It seems to be capable of acting on absorbed potassium in a rather consistent fashion. That this acid can break down primary potassium silicates or the more stable secondary minerals to an appreciable extent is doubtful. Citric acid undoubtedly attacks organic matter more than other solvents do and it may obtain some potassium from this source. Whatever its specific activity, the results which it yields

are more in accord with the results given by plants themselves than are some of the other methods used in these studies.

The use of steam under pressure in conjunction with water or citric acid yielded no better results than were obtained with these solvents at ordinary temperatures. It is possible that the pressure used was not sufficiently high, since the best results obtained in Germany were those in which a steam pressure of five atmospheres was attained. It is therefore unfair on the basis of this study to make a decision upon the merits of this method of attack.

Neubauer's method appears to be useful for indicating the available potassium of soils. Unfortunately the method is rather tedious. The choice of the crop to use may be questioned because of the known differences in feeding power of crops. Whether the results obtained with rye are suitable criteria for judging the needs of sugar beets, for instance, is not easily decided, and certain investigators are quite skeptical regarding the matter. Concordant results were obtained in this work with the rye in Neubauer tests and with alfalfa yields in regular greenhouse pot tests. In washing the soil out of the mass of plant roots with running water there is undoubtedly a loss of certain of the dead epidermal cells of the roots. Appreciable quantities of potassium may thus be lost. During the latter stages of growth, potassium salts are exuded by the leaf tissue and can be easily removed by washing, but it is not probable that there is danger of this with the younger seedlings. The soil is really worked over thoroughly by the large mass of roots developed by 100 seedlings, and competition is undoubtedly keen, a situation which should favor the extraction of potassium and other nutrients to a marked degree.

The use of the same arrangement of cultures as prescribed by Neubauer actually to test the response of plants to fertilization seems to offer a promising new means of attack which is worthy of attention. In the case of the three soils tested, the method agrees well with the results of regular greenhouse pot tests.

Leaching of soils with normal ammonium chloride solution appears to be one of the best ways of determining the amount of replaceable potassium present in soils. The quantities of potassium made soluble by this means are quite closely related to the quantities obtained by plants themselves. This is good proof that the replaceable potassium is the available potassium. The rate of replacement or solubility of the potassium in this solvent is almost the same in all of the soils studied, which strongly suggests that the same potassium compound furnishes the available potassium in the different soils. This compound is probably of a zeolitic nature.

In summing up the entire situation as brought out by these experiments, four methods appear to be outstanding in possible usefulness: The Nebauer test and its modification, the one per cent citric acid extraction, the replacement with strong ammonium chloride solution, and leaching with normal ammonium chloride solution gave results which correlated very well with one another. If these tests make possible reliable estimations of potassium needs,

they should prove very useful and valuable. The ultimate usefulness of any laboratory test must of course be decided by actual tests in the field.

SUMMARY

The object of this investigation was to study several methods for the extraction of the available potassium in soils. Both plants and weak solvents were used as extractants. Plants were employed in Neubauer tests. The solvents used included distilled water, carbonic acid, citric acid, ammonium chloride solution, and steam. All of these studies were conducted with a variety of soils. The results lead to the following conclusions:

1. The Neubauer test evidently detects quite accurately the potassium deficiencies of soils.
2. Neubauer tests modified to include fertilization were good indicators of the potassium needs of soils.
3. Extraction with water and carbonic acid did not give reliable indications as to the amount of available potassium in soils.
4. Of the solvents used, ammonium chloride solution most closely simulated plant activity in removing available potassium.
5. One per cent citric acid solution is not so good as ammonium chloride solution because it does not show sufficient differences between good and poor soils.
6. There seems to be no additional information acquired by the use of one per cent citric acid in conjunction with steam under pressure over the use of citric acid alone.
7. Steam alone does not give results which consistently show what the potassium requirements of a soil are.
8. With normal ammonium chloride solution the leaching method of extracting the available potassium of soils gives better results than the single extraction method. When a soil is leached with this solution, the rate of solubility of the potassium is a constant for some time, but eventually becomes much slower. The conclusion is drawn that the first leachings remove the easily replaceable potassium, after which the slower rate of solubility is connected with the potassium compounds of low solubility. A relationship between the potassium dissolved from a soil by the first 200 cc. of leachate and that dissolved by a liter was established. This relationship may be used as a rapid means of determining the total replaceable or available potassium in soils.
9. The similarity of the velocity constants for the solubility of the potassium in different soils with ammonium chloride solution points to the existence of an exchange complex of the same composition in all the soils studied.
10. The replaceable potassium is believed to be the easily available potassium. Plants may not utilize all of the replaceable form during one season, but the relative abundance of this form in different soils appears to be a good measure of the amount of readily available potassium in them.

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PLATE 1

FIG. 1. The growth of rye on soil No. 7 in Neubauer tests modified to include the fertilizers indicated.

FIG. 2. The growth of rye on soil No. 3 in Neubauer tests modified to include the fertilizers indicated.

FIG. 1

FIG. 2

UNIV. OF MICH.

THE FIXATION OF PHOSPHATE BY A PEAT SOIL[1]

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The fixation of phosphates by soil has been the subject of numerous investigations, but little of this work has dealt with soils containing a high percentage of organic matter. This investigation was undertaken, therefore, to determine some of the factors contributing to the fixation of phosphate by a peat soil.

Comber (3) in his review of the literature discusses the two theories of physical adsorption and chemical precipitation held by previous investigators. He is of the opinion that phosphate is fixed only when insoluble compounds are formed, and submits evidence to show that the Fe, Al, and Ca, depending on the acidity of the solution, are the main cations concerned.

Gordon and his co-workers (5, 7, 13), working with colloidal gels in ion and aluminum found that they absorbed more phosphate than silica gel and also that the pH of the medium was an important factor.

Working with Texas soils, Fraps (4) concludes that the fixation of phosphate is a function of the ion and aluminum content of the soil. Roszman (12) working with an electrodialyzed clay concludes there there is a physical adsorption of phosphate, with a maximum at pH 3.5. He assumes that all the iron phosphate would be soluble at this pH.

Teakle (14), after determining the solubility of various phosphatic compounds over a wide range of pH values, regards the cations Fe, Al, Mn, and Ca, as being responsible for the precipitation of PO_4 in the soil, depending on the pH of the medium. Although he states that there was some evidence of physical adsorption in the soils he worked with, he considered that it was of minor importance.

If the fixation is only a precipitation process, then it should be prevented by altering the pH so that the phosphate compounds would be soluble, or by leaching with a strong acid to remove the cations responsible for the precipitation. Evidence of the effect of any particular cation should be obtained by saturing the replaceable base complex with that cation and then determining the effect of an alteration of pH on the fixing power of the medium. If the cation in question was the sole factor, the soil should react in the same way as a

[1] Contribution from the plant nutrition laboratory, University of California. Acknowledgment is made to Prof. J. S. Burd for suggesting the problem and for advice and criticism during the investigation.

pure compound. The various experiments were planned with the aforementioned ideas in mind.

The peat used in this work was obtained from one of the islands in the delta of the San Joaquin River. It is composed almost entirely of Tule debris, pieces of undecomposed material being found at considerable depth. The particular sample used was from an area that had never been fertilized. It contained 72 per cent combustible material and had a reaction of pH 5.3. In the air-dry condition it contains about 18 per cent moisture. This peat, like that of many other places (1), requires the application of large amounts of phosphatic fertilizer and of these applications the major portion is immediately withdrawn from solution.

METHODS

The following method of procedure was adopted for all fixation experiments, the reagents being always added in the same order and the same proportions of peat and phosphate used in each case. In 200-cc. volumetric flasks, 10-gm. samples of peat were placed, 100 cc. of H_2O was added, then 400 cc. of a solution of KH_2PO_4 containing 0.5 mgm. of PO_4 per cc. To get the desired range of pH, varying amounts of HCl or NaOH were added to each flask. The contents were made up to volume, the flasks shaken several times, and let stand for about 18 hours. The solutions were then filtered on Büchner funnels through no. 42 Whatman paper. The first portion of the filtrate was returned to the funnel so that a clear solution was obtained. The pH of the resultant solution was determined electrometrically, a hydrogen electrode being used. Phosphorus was determined by precipitation as ammonium phospho-molybdate and titration. When the phosphate content was quite low, the coeruleo-molybdate method of Deniges as modified by Parker (10) was used.

Corrections were made for the moisture in the peat when the samples were weighed. The volume occupied by the peat was determined and deducted from the total volume when the total phosphate was computed. The ratio of 10 gm. peat to 20 mgm. phosphate was used in all cases where peat or peat extract was used. In reporting results, the amount of phosphate remaining in solution (unabsorbed) is plotted against the pH, mgm. phosphate being used as ordinates and pH values as abscissae.

The water-soluble salts were removed from a large quantity of peat by leaching on a Büchner funnel until the leachate gave no test for calcium or chloride. This material was then air-dried and used in the various experiments.

The solubility of the phosphorous in the aforementioned material was determined over a wide range of pH by making 1:5 extracts using solutions of HCl and NaOH of different concentrations. There was no added PO_4 in this experiment. The results are plotted in figure 1, the mgm. PO_4 dissolved from 10 gm. peat at the various pH values being shown by the curve.

The fixing power of the water-leached peat was determined by setting up a series of samples as outlined in the foregoing. The pH and the phosphate con-

tent of the resultant solutions were determined and plotted in figure 2, curve 1, which shows the amount of phosphate in solution at the different pH values.

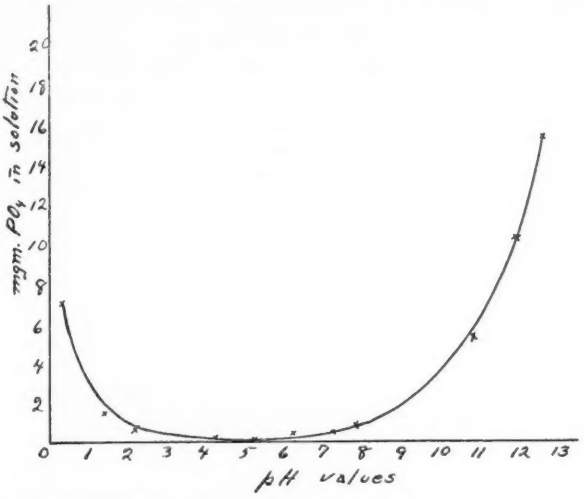

FIG. 1. PHOSPHATE DISSOLVED FROM 10 GM. WATER-LEACHED PEAT BY 1:5 EXTRACT USING HYDROCHLORIC ACID AND SODIUM HYDROXIDE OF VARIOUS CONCENTRATION

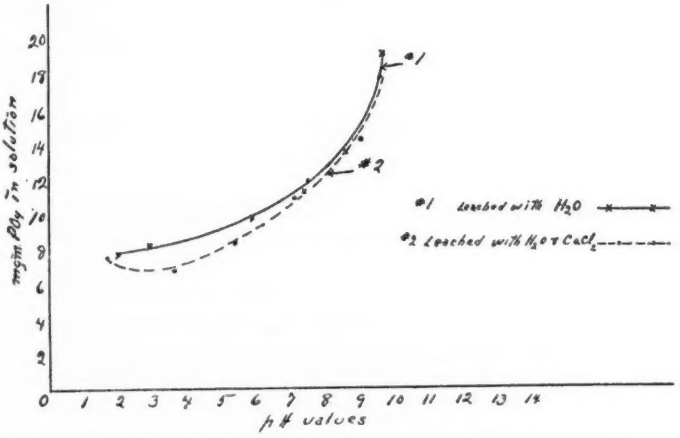

FIG. 2. FIXATION OF PHOSPHATE BY 10 GM. WATER-LEACHED PEAT

20 mgm. added phosphate

The curve shows a maximum fixation at pH 2 with a gradual increase in the amount of phosphate in solution as the alkalinity increased. This shows

either a hydrolysis of the compounds formed or a substitution of the OH ion for the adsorbed PO_4 ion. The latter explanation is in keeping with the work of Rona and Michaelis (11) on the adsorption of ions by charcoal, the OH ion being more strongly adsorbed than the other anions.

A similar series was set up using peat that had been leached with 0.05 *N*HCl according to the method of Gedroiz (6) for the purpose of removing the replaceable bases and saturating the complex with hydrogen. The peat was washed with water until the leachate gave no test for chloride. The effect of the acid treatment on the fixation of phosphate is shown in figure 3, curve 1. We find no fixation above pH6 and the maximum is near pH 1.5. In comparing

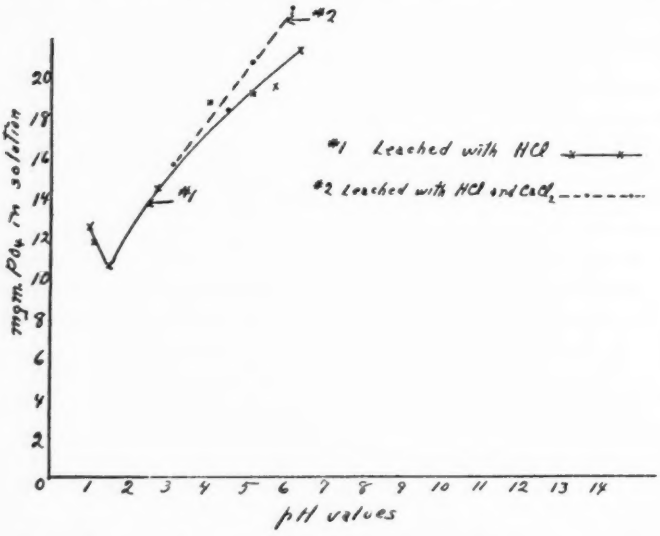

FIG. 3. EFFECT OF LEACHING PEAT WITH 0.5 NORMAL HYDROCHLORIC ACID
20 mgm. added phosphate

this with the water-leached peat (figure 2, curve 1), we find a marked decrease in the power of fixation, except around pH 2, due to the acid treatment. The increased phosphate content above pH 6 was accompanied by an increased solubility of organic material and is apparently connected therewith. The results show that either the fraction responsible for the fixation has been remove or its power destroyed. As the acid treatment would tend to remove most of the active cations, the evidence points toward a physical adsorption.

Having shown that leaching with acid depressed the power of fixation, we next tried to restore this power. Accordingly, a portion of the acid-leached peat was leached with *N* $CaCl_2$ using the proportions of 40 cc. of solution per gram of peat. The material was washed with water until free of chloride, air-

dried, and a series set up. The results are shown in figure 3, curve 2. Either the calcium was unable to replace the H ion or calcium in the replaceable base complex has no effect, for we find on comparing with curve 1 that the calcium treatment has had no appreciable effect on the fixation of phosphate.

Another series was set up using water-extracted peat in which the replaceable base complex has been saturated with Ca as in the previous experiment. Figure 2, curve 2 shows the results obtained. On comparing these results with figure 2, curve 1, the water-leached peat, we find that the treatment with calcium chloride has had no effect. In this case there can be no doubt about the replaceable base complex being saturated with

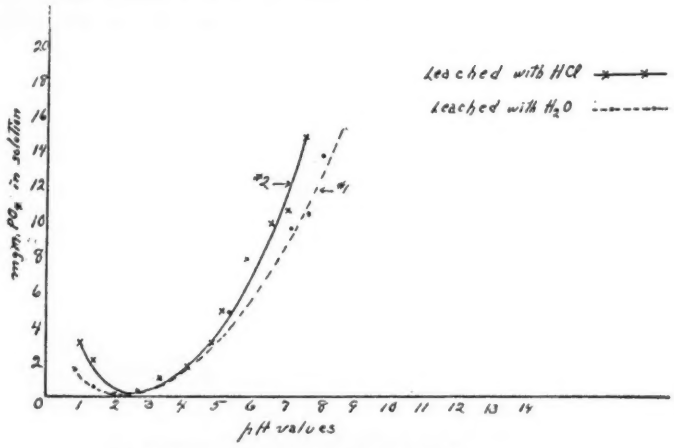

FIG. 4. EFFECT OF LEACHING PEAT WITH FERRIC CHLORIDE

20 mgm. added phosphate

calcium. As this soil contained water-soluble calcium before being leached it is possible that it was already saturated with calcium, in which case the treatment with calcium chloride would have but little effect. However, there is no evidence in the curve that calcium is functioning as a precipitating agent, because the amount of phosphate in solution tends to increase in the range of pH in which calcium phosphate should be precipitated (2, 14).

The effect of saturating the peat with iron and aluminum was then determined. Portions of the water-extracted and hydrochloric acid-extracted peat were leached with *N* $FeCl_3$ as in the treatment with $CaCl_2$. The peat was washed with water until the leachate gave no test for chloride. The fixing power of the iron-saturated material was then determined and the results are plotted in figure 4, curve 2 representing material that was leached with HCl. In both cases the result is the same—the amount of phosphate fixed has been materially increased (compare fig. 4 with figs. 2 and 3). At pH 2.5 there is

approximately complete fixation of the added phosphate, and a decrease with change of pH in either direction. The increased solubility with increased alkalinity indicates, as suggested by Teakle (14), the hydrolysis of the ferric phosphate and the formation of the more insoluble ferric hydroxide. As the calcium in the replaceable base complex has no appreciable effect on the fixation of phosphate (as shown by previous experiments) we would assume that the iron held in a similar form would also be ineffective. The small amount of iron held in the replaceable base complex indicates that it has changed into the more insoluble ferric hydroxide and it is possibly this form that is effective in fixing the phosphate. In the preceding experiment, we would expect a hydrolysis of the ferric chloride and a formation of ferric hydroxide, which would be held in the peat. This freshly precipitated hydroxide would be readily

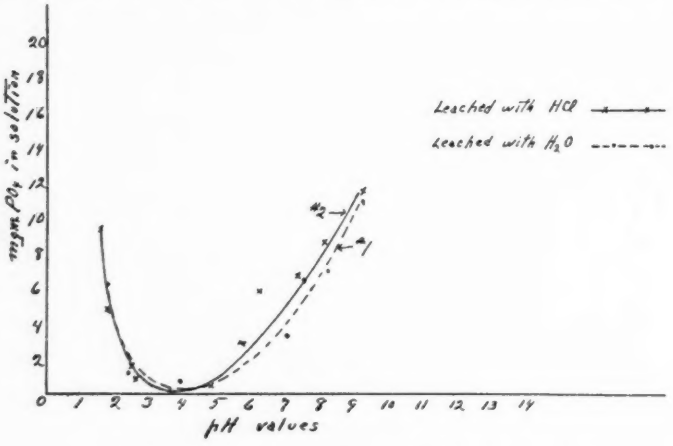

FIG. 5. EFFECT OF LEACHING PEAT WITH ALUMINUM CHLORIDE

20 mgm. added phosphate

soluble in an acid reaction and would tend to precipitate the phosphate. It is impossible to determine whether the retained iron is held by the replaceable base complex, or merely as precipitated ferric hydroxide, but the evidence seems to be in favor of the latter.

Similar results were obtained when portions of water and hydrochloric acid leached peats were saturated with aluminum by leaching with *N* $AlCl_3$. The previous treatment with water or hydrochloric acid had but little effect, as shown in figure 5. The maximum fixation is at pH 4.5 with an increased solubility in both directions from this point. The degree of hydrolysis is not quite so great as in the case of the iron. The same questions arise as to how the active aluminum is held in the peat and the same conclusions must be drawn. Whatever the form of the iron and aluminum in the soil, however, they are very effective in fixing phosphate.

Having found that leaching the peat with ferric or aluminum chloride would increase its power of fixation in acid reactions, the author considered it neces-

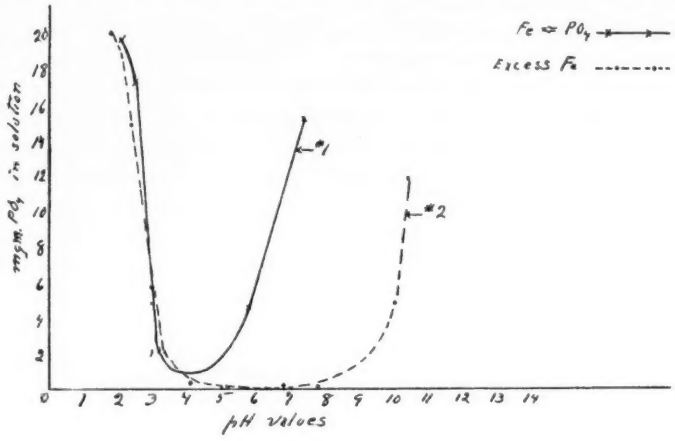

FIG. 6. SOLUBILITY OF FERRIC PHOSPHATE

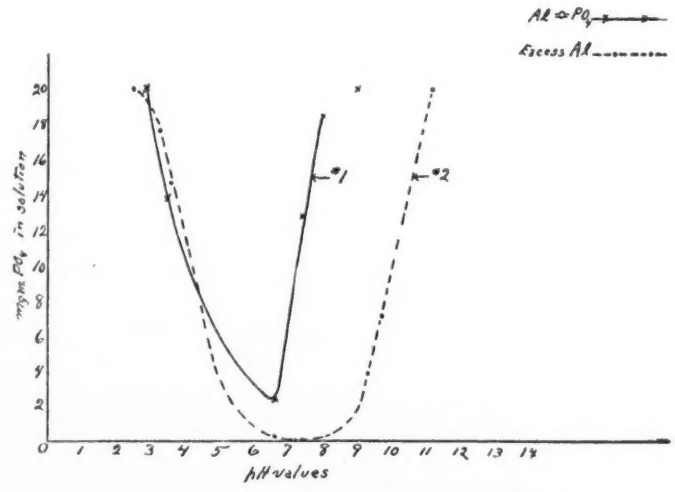

FIG. 7. SOLUBILITY OF ALUMINUM PHOSPHATE

sary to determine the solubility of ferric and aluminum phosphate at various pH values.

From a solution of known concentration of ferric chloride, aliquots were taken containing iron equivalent to 20 mgm. phosphate. These were placed in

200-cc. volumetric flasks and 20 mgm. phosphate added from the standard solution of potassium dihydrogen phosphate. The pH was adjusted as in previous experiments, and the volume made up to the mark with water. After standing the usual time the solutions were filtered and the pH and phosphate determined as for the other experiments. A similar series with the ratio of 10 equivalents of iron to 1 of phosphate was set up to determine the effect of excess iron. The method of procedure was the same as in the previous experiment. The results of the two series are plotted in figure 6. In both curves we find all the phosphate in solution at pH 2 and practically none at pH 4. Where the ratio

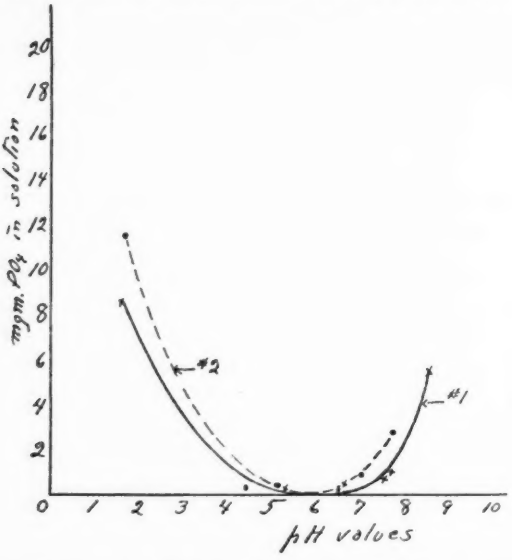

FIG. 8. EFFECT OF ADDING 50 CC. 0.25 NORMAL HYDROCHLORIC ACID AND 20 MGM. PHOSPHATE TO 10 GM. PEAT

Curve 1 represents acid in contact with peat for 30 minutes
Curve 2 represents acid in contact with peat for one week

$Fe/PO_4 = 1$ (curve 1), there is a hydrolysis of the ferric phosphate starting at pH 4, and a very rapid hydrolysis above pH 6. Where the ratio $Fe/PO_4 = 10$ the solubility of the phosphate was depressed until about pH 10 after which a rapid hydrolysis occurred.

On the alkaline side of pH 4, curve 1 agrees closely with the curves in figure 4, where the peat was leached with ferric chloride. On the acid side some other factor is depressing the solubility of the phosphate where the peat is present. This may be caused by physical adsorption, as shown in figure 3, curve 1. Where the peat and Fe are both present there is a combined effect.

Two series were also set up using aluminum chloride in the same ratios

as the ferric chloride in the previous experiment. The results are shown in figure 7. In both series we have a maximum precipitation at pH 6.5. The effect of the increased ratio of Al/PO_4 was similar to that where the ferric phosphate ratio was increased but it did not extend over so wide a range of pH. Miller (9) claims the formation of the definite compound, $AlPO_4$, from the time precipitation starts at pH 3 up to pH 4.5, after which the ratio of Al/PO_4 increases. Around pH 7 aluminates are formed which dissolve at higher alkalinity. In comparing the curves with those for the aluminum chloride-treated peat (figure 5) we do not find the same agreement as with the Fe. Although the aluminum has a marked effect on the fixation of phosphate by the peat, the results are not in accord with the aforementioned curves, indi-

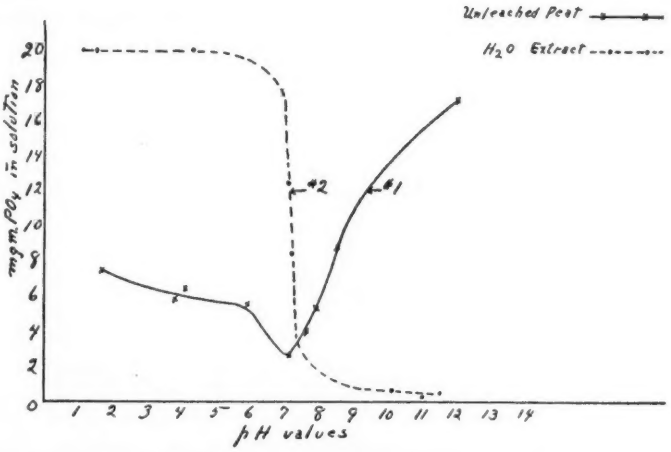

FIG. 9. FIXATION OF PHOSPHATE BY UNLEACHED PEAT AND BY WATER EXTRACT
20 mgm. added phosphate

cating that the controlling factor in the peat is not reacting as an $AlPO_4$ compound.

If iron and aluminum were functioning as precipitating agents in the peat, an increase in the amount of soluble iron and aluminum should increase the fixation of phosphate.

Ten-gram samples of water-leached peat were placed in the volumetric flasks and 50 cc. of 0.25 *N* HCl was added for the purpose of bringing more iron and aluminum into solution. The phosphate solution was then added, the hydrochloride neutralized, and the pH adjusted as for the previous series.

A second series was set up in which the acid was left in contact with the peat for one week before the phosphate was added. Otherwise the two series were the same. The pH and phosphate content of the resultant solutions are plotted

in figure 8. The two curves are very similar, showing that the increased time did not bring any more material into solution. The power of fixation has been materially increased by the acid treatment when the products of the reaction were not removed. (Compare these results with figure 3, curve 1, where the peat was leached with hydrochloride.)

A series was set up using the unleached peat in the same proportions as for previous series. In addition to the usual determinations of pH and phosphate, the calcium in the resultant solutions was determined. The amount of phosphate remaining in solution at the various pH values is shown in figure 9. In comparing these with the curve for the water-leached peat (figure 2, curve 1), we find that the water-soluble salts are exerting considerable effect between pH 6 and 8. This lowering of the curve is apparently caused by the formation of calcium phosphate (2, 14). The calcium in solution in each sample is listed in

TABLE 1

Phosphate and calcium in solution at various pH values

(10 gm. unleached peat + 20 mgm. PO_4)

pH	PO_4	Ca
	mgm.	mgm.
1.7	7.4	165.0
3.8	5.9	46.9
5.9	5.5	18.3
7.1	2.6	8.4
7.6	3.9	7.9
7.9	5.2	9.8
8.5	8.7	24.6
12.0	17.2	72.9

table 1, which shows that sufficient calcium was present at all times to precipitate all the soluble phosphate.

Apparently the presence of the peat prevents the precipitation of the phosphate by calcium, for the analysis of the various samples in the series in table 1 show sufficient Ca present to precipitate the phosphate.

The fixing power of the aqueous phase when removed from the peat was determined by leaching a sample of peat with water until the leachate gave no test for calcium or chloride. Aliquots of this solution representing the leachate from 10 gm. of peat were used for setting up a series of samples. The usual 20 mgm. of phosphate was added and the pH adjusted. The results are shown in figure 9, curve 2. The water-soluble salts had no effect until pH 6 was reached, then complete precipitation of phosphate took place, but no hydrolysis was evident as when the peat was present. The pH at which precipitation took place shows that calcium is causing it. There might be some effect from the formation of magnesium phosphate, which is also precipitated in this range. An analysis of the leachate showed that it contained more than enough calcium

to cause the precipitation of all the phosphate as calcium phosphate. The important point is that when removed from the peat, the water-soluble salts caused the complete precipitation of the phosphate instead of throwing out only a few milligrams as in the presence of the solid material.

Further evidence of the effect of organic material on the calcium was obtained by treating 10-gm. samples of unleached peat with 50 cc. of .25 *N* HCl, thus bringing an equal amount of calcium into solution in each sample before the phosphate solution was added. After the phosphate was added the solutions were neutralized with NaOH and the pH was adjusted.

The phosphate and calcium content and the pH were determined and the results are reported in table 2.

We find that a large amount of the calcium has been thrown out of solution before reaching a pH at which the formation of calcium phosphate would take

TABLE 2

Phosphate and calcium in solution at various pH values

(10 gm. unleached peat + 50 cc. .25*N* HCl and 20 mgm. PO_4)

pH	PO_4	Ca
	mgm.	*mgm.*
1.7	8.4	132.8
2.6	5.0	124.8
3.3	3.2	68.8
4.4	2.0	49.6
5.8	1.3	25.6
7.0	1.3	12.0
7.4	2.4	8.6
8.3	4.2	17.6
8.7	5.6	20.8
9.5	10.0	39.2

place. In fact the calcium in solution is still decreasing after the phosphate has started to increase, and at more alkaline pH values we find an increase in both calcium and phosphate. The removal of calcium from solution on the acid side of pH 5 is apparently caused by the formation of insoluble compounds of calcium and organic material which is hydrolyzed when the alkalinity is increased above pH 8. The fact that the calcium does not precipitate all the soluble phosphate on the alkaline side would suggest that either the calcium or phosphate or both were held in some soluble form that did not ionize.

A study was now made of the hydrochloride extract. A sample of unleached peat was leached with 0.05*N* HCl until the leachate gave no test for calcium. Aliquots of the leachate representing 10 gm. of peat were placed in flasks and 20 mgm. of phosphate was added in the same manner as where peat was used. In a second set of aliquots of the leachate the iron and aluminum were precipitated by ammonium hydroxide. The solutions were filtered and the precipi-

tate was washed free of chloride. The precipitate from each aliquot was used as an individual sample for one series, whereas the combined filtrate and washings from each aliquot were used as an individual sample for another series. The results of three series are plotted in figure 10. Where the samples contained all of the dissolved material (curve 1), the phosphate was all precipitated at pH 5 and it remained out of solution. In curve 2, where we have only the iron and aluminum, the phosphate is practically all precipitated at pH 3.5 but goes into solution again above pH 7. In curve 3 there is no precipitation until pH 6.7 is reached, when the calcium becomes effective, causing the complete precipitation of the phosphate. In these three curves we have the com-

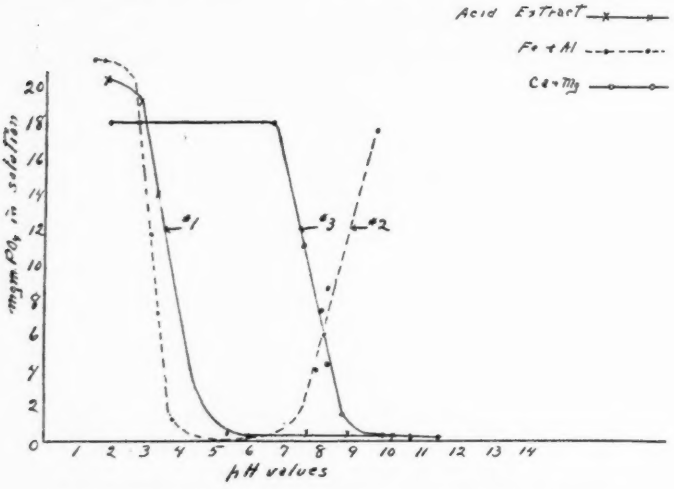

FIG. 10. FIXATION OF PHOSPHATE BY HYDROCHLORIC ACID EXTRACT

20 mgm. added phosphate

bined effect of all factors and also the effect of the iron and aluminum as compared with the other cations.

The calcium is functioning in the same way in the acid extract as in the water extract, being able to precipitate all of the added phosphate when removed from the solid phase, showing again that the peat exerts a depressing effect on the activity of the calcium.

SUMMARY

In considering the data presented, it is evident that precipitation and physical adsorption are both functioning in the removal of phosphate from solution. Although the formation of iron, aluminum, and calcium phosphates will account for the fixation above pH 2 there is considerable fixation below this point.

Leaching with twentieth-normal hydrochloric acid, which should remove all of the reactive iron, aluminum, and calcium, caused a loss of the power of fixa-

tion, except below pH 5. Any fixation that took place in the acid-leached material must have been due to physical adsorption.

Only water-soluble calcium was able to precipitate phosphate in the presence of peat, the calcium in the replaceable base complex having no effect even when the complex was saturated with it. The presence of the organic material prevented some of the water-soluble calcium from uniting with phosphate, for there was soluble calcium and phosphate present at the same time in an alkaline medium (table 1).

Leaching the peat with either ferric or aluminum chloride caused a decided increase in the amount of PO_4 fixed. This method was equally effective, despite the previous leaching of the peat with water or hydrochloric acid.

A study of the solubility of ferric phosphate at various pH values indicated a behavior somewhat similar to that of peat leached with ferric chloride.

There is considerable evidence that the solid phase material exerts an appreciable effect upon the fixation of phosphate in all cases.

The various cations when removed from the peat by leaching with water or hydrochloric acid react in the same way when phosphate is added as do the pure compounds of the aforementioned ions.

In the material studied the formation of iron, aluminum, and calcium phosphates will account for the fixation of phosphate under field conditions.

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CAN BACTERIUM RADICICOLA ASSIMILATE NITROGEN IN THE ABSENCE OF THE HOST PLANT?

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The question of whether *Bacterium radicicola*, when cultivated in a nutrient medium in the absence of the host plant, is able to fix atmospheric nitrogen is an old one and has as frequently been answered in the positive as in the negative sense. As an exhaustive survey of the literature on this subject has been compiled recently by Hopkins (10), only those papers will be discussed here which bear directly on the side of the question treated in this paper.

Investigators agree that to obtain a good growth of the organism in pure culture, the nutrient medium must contain nitrogen in some form initially. Although it is possible to grow it on a medium to which nitrogen has not been added, few colonies occur and the growth is meagre (3, 4, 9, 11). The usual method of proving any nitrogen fixation by growing the organism studied on a nitrogen-free medium and assuming the nitrogen determined in the end of the experiment to be the nitrogen assimilated, is thus out of the question. So the difference between an initially higher amount of nitrogen and the amount estimated in the end must be determined. In the author's opinion it is here that the chief source of error and confusion may lie.

The media in use for the experiments on nitrogen fixation by *Bacterium radicicola* may be divided into two groups; namely, plant extracts containing a high amount of nitrogen, and soil extracts and synthetic media much poorer in nitrogen.

Beijerinck (3) was the first to introduce extract of pea foliage as a nutrient medium and since then many extracts of leguminous plants have come into use. Although the first determinations by Beijerinck in 1888 on the ability of

[1] This investigation was begun in the department of agricultural bacteriology of the University of Wisconsin, Madison. Many thanks are due for the conveniences of the laboratory put at my disposal, and I am greatly endebted to Dr. E. B. Fred for much valuable help and advice given. The investigation was brought to a close in the Technical University of Delft (Holland). The organisms were cultivated in the Laboratorium voor Microbiologie. I thank Dr. A. J. Kluyver of that laboratory for the interest taken in the progress of the investigation. The chemical work was carried out in the Laboratorium voor Analytische Scheikunde. I am especially endebted to Prof. H. ter Meulen, who gave me his personal help in the handling of his method and who put at my disposal apparatus and chemicals such as are used by him for his own estimations. His valuable suggestions have greatly helped me in bringing the investigation to a close.

nodule organisms to fix nitrogen were carried out on a medium of nutrient salts with and without the addition of asparagine (experiments which gave a negative result), his use of pea foliage extract (5) in later years produced the same result.

Mazé (14) was the chief advocate of the fixation of nitrogen in very large amounts. He used an extract of bean seeds with 2 per cent sucrose as a substrate. With a nutrient medium containing initially 44.8 mgm. of nitrogen in 100 cc. of medium[2] he got an increase of 46.4 mgm., an increase many times greater than any noted by later investigators on the same or any other media. Many later authors have pointed to the cheese odor of the cultures mentioned by Mazé, as being the weak point of this experiment, since it renders the purity of the cultures used at least doubtful. Mazé states clearly that the determination of the initial content of nitrogen was carried out at the opening of the experiment.

Golding (7) used pea foliage extract as a culture medium and made use of a special device to filter dissimilation products out of the cultures. He notes a nitrogen increase of 2.1 and 3.5 mgm. When his controls were analyzed is not stated.

Gino de Rossi (19) arrives at very irregular results with bean foliage and bean seed extracts. Since the checking of the blanks is very unsatisfactory (a difference in two determinations as high as 27 mgm.) no conclusion can be drawn out of a loss of 63.4 mgm. and an increase of 21 mgm. in inoculated cultures. The paper was not available, therefore when his controls had been analyzed could not be determined.

Olaru (18) in studying the effect on nitrogen fixation of the addition of varying doses of manganese sulfate to Mazé's medium get an increase up to 75.5 per cent of the initial nitrogen content (42.5 mgm.). But here again the time of analysis of the controls has not been stated.

Voicu (21), although considering the evidence of Mazé conclusive, studied the influence of the addition of boron to the medium. He obtained an increase of 2.2 to 3.8 mgm., the initial content of the medium being 13 to 17 mgm. Since the paper was not available, the time of analysis of the controls could not be determined.

Evidently from the existing data for plant extracts no definite conclusion can be drawn as to the ability of *Bacterium radicicola* to fix nitrogen.

Among the media with a low initial content of nitrogen, the chief one is soil extract.

In 1905, Löhnis (13) used this medium as a substrate and many others have followed his technique. All in all, 14 estimations are reported by him—the highest amount of nitrogen fixed being 1.54 mgm., the lowest 0.14 mgm., and one culture remained on the same level as the controls. Löhnis states that the analysis of his controls was carried out at the close of the experiment. Besides

[2] In reviewing the literature the amount of nitrogen has always been calculated on the basis of 100 cc. of culture medium, in order that the figures may be directly comparable.

B. radicicola he put other organisms, *B. radiobacter*, *B. pneumoniae*, *B. lactis viscosum*, and *B. prodigiosum* to the test, and notes for all an increase of about the same size as for *B. radicicola; B. fluorescens* and *B. agreste*, however, which show a very poor growth, give hardly any increase in nitrogen.

In 1912 (6) Fred reported his results obtained on soil extract medium. In a liquid medium with an initial content of nitrogen of 5.5 mgm. the amounts of increase varied between 0,76 to 1.25 mgm.; on an agar substrate containing initially 4.42 mgm. the amounts varied from 0.90 to 1.60 mgm.

Joshi (12) using the same substrate found an average increase in his cultures of 1.4 mgm. and a maximum of 2 mgm. He analyzed his controls at the close of the experiment.

Purely synthetic media have been used in the following investigations. Moore (16) reports that on a medium containing magnesium sulfate, potassium phosphate, and maltose, with an initial content of nitrogen of 0.3 mgm. an actual gain varying from 0.2 to 2.2 mgm. was found. When ammonium phosphate had been added the gain was greater. In the former experiments, analyses of the controls were made in the beginning; for the latter, the time of analysis was not recorded.

Hills (9) made use of mannitol agar, adding to the initial medium nitrates in varying amounts. The increase of cultures over controls varied between 0.15 and 3.50 mgm. It was higher when some nitrate had been added to the medium. The controls were analyzed at the close of the experiment.

Barthel (2) used a mannitol medium, adding 0.02 per cent KNO_3. He could not record any fixation of nitrogen. As far as could be made out, his controls were analyzed at the beginning of the experiment.

In a quite recent paper of Hopkins (10) which I was enabled to read before its publication, attention is drawn to the fact that discrepancies in results may be explained by the presence of nitrates in the nutrient medium. The author points to the fact that when the Kjeldahl method is used, it can be conceived that the nitrate nitrogen might escape detection. This nitrate, however, when converted by bacterial action into albuminous substances, would be recovered, and any increase of nitrogen noted as a result of this might be wrongly ascribed to a fixation of atmospheric nitrogen.

CULTURES

Strains out of five different inoculation groups were tested as to their ability to fix nitrogen. All cultures had been repeatedly tested as to their ability to induce nodule formation, and their purity was regularly controlled by litmus milk and potato tests.

The record of their isolation is given in table 1. In the numbering, the registration of the laboratory in Madison (Wis.) has been followed. The strains Pea 15 and Red Clover 202 are abnormal in their behavior. Although they are perfectly able to induce nodule formation, it appears when the host plants are grown in nitrogen-free sand that these plants do not surpass the

uninoculated controls in development. Apparently, therefore, these strains cannot supply the host plants with nitrogenous substances. The behavior of pea strain Pea 15 has been described in detail by Helz, Baldwin, and Fred (8).

COMPOSITION OF MEDIA

1. *Extract of pea foliage* was made up of 300 gm. of green, young pea plants (just before blooming) and 1000 cc. of tapwater. This mixture was heated to boiling and held for four hours on a water bath at approximately 100°C., filtered, and the filtrate made up to the original volume. One per cent of sucrose was added. The extract stained blue with diphenylamine sulfuric acid.

2. *Extract of bean seeds* was made by soaking 250 gm. of beans (*Phaseolus vulgaris*) in 500 cc. of tapwater for two hours. The water was then poured

TABLE 1

General description of cultures of Bacterium radicicola

NAME	NUMBER	SOURCE OF ORIGIN	DATE OF ISOLATION
Pea—*Pisum sativum*	14	Nodule, Delft, Holland	1926
Pea—*Pisum sativum*	3	Nodule, Madison, Wis.	1921
Pea—*Pisum sativum*	15	Vetch, *Vicia sativa*	1924
Red clover—*Trifolium pratense*	205	Commercial culture	1922
Red clover—*Trifolium pratense*	202	Nodule, Madison, Wis.	1921
Dalea alopecuroides	901	Nodule, Madison, Wis.	1924
Broad bean—*Vicia faba*	321	Nodule, Delft, Holland	1926
Bean—*Phaseolus vulgaris*	402	Nodule, Madison, Wis.	1924
Soybean—*Soja max*	501	U. S. Dept. Agr.	1918
Sweet clover—*Melilotus alba*	115	Nodule, Madison, Wis.	1922

off and the beans steamed in 2000 cc. of tapwater for two hours. This extract was then filtered and 1 per cent of sucrose added.

3. *Yeast-water mannitol medium* was composed of: Mannitol, 15 gm.; K_2HPO_4, 0.5 gm.; $MgSO_4$, 0.2 gm.; NaCl, 0.2 gm.; $CaCO_3$, 3.0 gm.; Yeast-water, 100 cc.; Tapwater, 900 cc.

4. *Soil extract.* The soil was mixed with one and one half times its weight of water and autoclaved for 30 minutes at 10 pounds steam pressure. Calcium carbonate was added to this suspension and then the clear liquid filtered off. One per cent of the desired carbohydrate and 0.05 per cent K_2HPO_4 were then added. The soil extract as it has been used in Madison (Wis.) gave a positive reaction with the diphenylamine test; as it has been used in Delft, a negative.

EXPERIMENTS CARRIED OUT IN MADISON (WIS.)

Cultural methods

Culture media in 25-cc. portions were filled into flat 375-cc. bottles. In this way a thin layer of medium about 1 cc. deep was obtained. After the

bottles were sterilized about 0.5 gm. of sterile $CaCO_3$ was added to each bottle. The inoculations were made by adding three drops of a suspension of bacteria in sterile water.

Since a small increase in the nitrogen content on incubation might be due to the taking in of nitrogenous compounds from the atmosphere, an experiment was carried out with purified air. For this purpose 50 cc. of the medium was pipetted into 750-cc. Erlenmeyer flasks. The flasks were connected in series and a uniform air current was passed through. The air was freed of nitrogenous substances by passing through scrubbing towers of acid and alkali.

Nitrogen analyses were made according to the official Gunning Arnold method and according to the modification including nitrates, where before destruction salicylic acid acts upon the substance (1, p. 8). The pea foliage extract, especially, made difficult a close checking of the uninoculated controls, and many experimental series had to be discarded on this account. Analysis of the controls were made after they had been kept incubated together with the inoculated cultures. The results obtained later in Delft indicate clearly

TABLE 2

Nitrogen content of 100 cc. of pea foliage extract

DETERMINED ACCORDING TO GUNNING ARNOLD METHOD		DETERMINED ACCORDING TO MODIFICATION FOR INCLUDING NITRATES	
Individual	Average	Individual	Average
mgm.	*mgm.*	*mgm.*	*mgm.*
50.36	49.36	50	50.20
48.36	.....	51.50	.....
49.76	.....	49.75	.....
48.96	.....	49.75	.....

that a comparison should have been made of the nitrogen content of the controls at the opening and the close of the experiments.

The nitrogen content of the pea foliage extract when determined according to the Gunning Arnold method was slightly less than when determined by its modification where salicylic acid had been added to the liquid (table 2). The differences in the nitrogen content of inoculated bottles and controls were the same, however, by both methods.

The soil extract medium has always been analyzed according to the salicylic acid method, including nitrates.

Since a conclusive settling of the question of nitrogen fixation in the positive sense appeared likely, numerous experiments with different nutrient media and different strains were carried out. From the numerous data at hand some will be cited as examples.

Pea foliage extract

Data obtained on pea foliage extract are given in table 3. It may be noted that on this medium Pea strains, as well as the Dalea strain from another cross-

TABLE 3

Nitrogen fixation in pea foliage extract calculated on 100 cc. of medium

TIME OF INCUBATION	N IN CONTROLS		STRAIN NUMBER	N COMPARED WITH CONTROLS	
	Individual	Average		Individual	Average
days	*mgm.*	*mgm.*		*mgm.*	*mgm.*
10	43.33	43.12	Pea 14	+4.21	+3.31
	44.03			+3.31	
	41.89			+2.41	
	43.93				
	43.49		Pea 3	+1.81	+2.26
	42.73			+1.61	
	42.53			+2.61	
				+3.01	
			Bean 402	+2.00	+2.40
				+2.81	
			Dalea 901	+1.71	+2.46
				+3.05	
				+2.61	
			Broad bean 321	−0.09	+1.48
				+2.91	
				+1.91	
			Sweet clover 115	−0.59	−0.31
				−0.03	
			Soy 501	−0.95	−0.76
				−0.89	
				−1.39	
				+0.17	

TABLE 4

Nitrogen fixation on pea foliage extract, diluted from 165 mgm. N, calculated on 100 cc. of medium

TIME OF INCUBATION	N IN CONTROLS		STRAIN NUMBER	N COMPARED WITH CONTROLS	
	Individual	Average		Individual	Average
days	*mgm.*	*mgm.*		*mgm.*	*mgm.*
8	29.03	28.29	Pea 14	−2.06	−2.18
	27.73			−1.46	
	27.95			−0.16	
	27.33			−1.46	
	28.43			−3.66	
	29.03			−2.10	
	28.53				

inoculation group, show a distinct increase in nitrogen content when the cultures are compared with the uninoculated controls at the close of the experiment.

In other experimental series the same results were obtained, with the exception of one series in which the pea foliage extract, as the result of longer boiling, was three times as high in nitrogen content as usual. Although this extract

TABLE 5

Nitrogen fixation in bean seed extract calculated on 100 cc. of medium inoculated by strain Pea 14

TIME OF INCUBATION	N IN CONTROLS		TIME OF INCUBATION	N COMPARED WITH CONTROLS	
	Individual	Average		Individual	Average
days	*mgm.*	*mgm.*	*days*	*mgm.*	*mgm.*
		Experiment 1			
7	31.69	31.63	9	+3.75	+3.59
	31.83		9	+3.39	
	33.33		9	+5.35	
	30.63		9	+4.31	
	30.43		15	+2.13	
	32.63		15	+2.93	
	32.03		15	+3.21	
	32.03				
	32.03				
		Experiment 2			
6	33.12	33.32	6	+0.44	+0.33
	33.20		6	+0.34	
	33.16		6	−0.06	
	33.26		6	+0.40	
	32.96		6	+0.10	
	33.76		6	+0.48	
	33.50		6	+0.44	
	33.66		11	+0.69	−0.20
	33.26		11	−0.21	
	33.32		11	+0.39	
			11	−0.67	
			11	−0.71	
			11	−0.41	
			11	−0.51	

had been diluted to the concentration usually studied, the same strain, Pea 14, which had been tested out frequently here gave constant negative results (table 4). When this experiment was conducted, no explanation of this phenomenon was arrived at, and conclusions were drawn from all the other experiments in which increases had constantly occurred.

Bean seed extract

A similar irregularity was encountered when the bean seed medium, as used by Mazé, was tried out. Strain Pea 14 was used again as inoculation material. Table 5 gives the results of two series of experiments carried out with bean seed extract made up separately for both the series. Although in the first series a constant increase of nitrogen occurred in all of the inoculated bottles, nothing like this was found in the second series, which was set up for comparison. The results with this medium, therefore, were very irregular and in no case were the very high amounts of nitrogen fixed as reported by Mazé.

TABLE 6

Nitrogen fixation in soil extract with the addition of 1 per cent glucose, calculated on 100 cc. of medium

TIME OF INCUBATION	N IN CONTROLS		STRAIN NUMBER	N COMPARED WITH CONTROLS	
	Individual	Average		Individual	Average
days	*mgm.*	*mgm.*		*mgm.*	*mgm.*
15	4.42	4.13	Pea 14	+1.85	+1.62
	3.92			+1.39	
	4.06				
			P ea 3	+1.13	+0.99
				+0.85	
			Pea 15	+1.03	
			Red clover 205	+0.69	+1.42
				+1.85	
				+1.73	
			Red clover 202	+1.65	+1.62
				+1.59	
			Bean 402	+1.73	+1.37
				+1.26	
				+1.09	
			Dalea 901	+1.99	+1.69
				+1.39	

Yeast-water mannitol medium

In testing various strains on this medium no fixation could be reported.

Soil extract

This medium having been used by many investigators and generally with slightly positive results, it was tried out with the addition of one per cent of either sucrose, glucose, or mannitol. Here again the comparison of the cultures with

the controls at the close of the experiments for different strains gave a constant increase of nitrogen in the former, in no way lower than had been reported by former investigators, in fact often higher. Experiments carried out with purified air gave the same results.

Table 6 gives the results of one typical experiment.

In table 7 the results of all experiments carried out with soil extract and pea foliage extract are combined, with the exception of the figures for the pea foliage extract diluted down from higher concentrations, mentioned in the foregoing.

Although different culture media gave different results, it was considered proved that many strains of the nodule organisms were able to fix a uniform although low amount of nitrogen under the conditions chosen.

TABLE 7

Comparison of the increase in nitrogen induced by various strains

STRAIN	SOIL EXTRACT MEDIA				PEA FOLIAGE EXTRACT MEDIA			
	Number of cultures showing		Gain or loss in nitrogen		Number of cultures showing		Gain or loss in nitrogen	
	Gain in nitrogen	Loss in nitrogen	Maximum	Average	Gain in nitrogen	Loss in nitrogen	Maximum	Average
			mgm.	*mgm.*			*mgm.*	*mgm.*
Pea 14	16	2	2.26	1.06	23	0	4.21	2.54
Pea 3	2	0	1.13	0.99	6	0	3.01	2.09
Pea 15	5	0	2.30	1.37	1	3	2.41	−0.43
Red clover 205	18	0	3.24	1.75	....	....	....	.....
Red clover 202	2	0	1.65	1.62	....	....	....	.....
Bean 402	12	2	3.19	1.38	6	0	2.81	1.58
Sweet clover 115	7	3	2.40	0.74	0	5	....	−1.18
Soybean 501	3	6	2.00	−0.27	1	3	0.17	−0.76
Dalea 901	17	0	3.33	2.13	5	0	3.05	2.18
Vicia faba 321	....	....	....	....	2	1	2.61	1.48

EXPERIMENTS CARRIED OUT IN DELFT (HOLLAND)

As further general research was carried out on both the non-working strains Pea 15 and Clover 202, more numerous data were required on the nitrogen fixation ability of these strains. This work was carried out in Delft (Holland). As only a few Kjeldahl determinations could be carried out simultaneously, the ter Meulen method (15, 20) was used for the estimation of nitrogen. This proved to be very accurate, and therefore well-suited to this investigation. Some recent modifications of this method (17) which have been applied in this investigation consist in the following:

Instead of reduced nickel, nickel formiate was added to the liquid to be tested out in the porcelain boat. The nickel formiate, dissolving in the liquid as a result of the heating, thoroughly impregnates the substance tested. As the result of further heating the nickel is

reduced, and thus a very thorough mixture of the substance tested, with very finely divided active nickel is obtained.

The ammonia is taken up in a U-tube, the form of which is shown in figure 1. In this U-tube are placed one drop of methyl orange indicator and one drop of the acid. In the course of the experiment as soon as the color changes, a new drop of acid is added. Thus the amount of liquid in which the titration is carried out is brought down to a minimum. In this small volume of titration liquid, the color change of the methyl orange shows very distinctly. When the titration is carried out with 0.01 *N* acid and a burette is used on which 0.001 cc. may be estimated, the titration error need not surpass 0.002 mgm. of nitrogen.

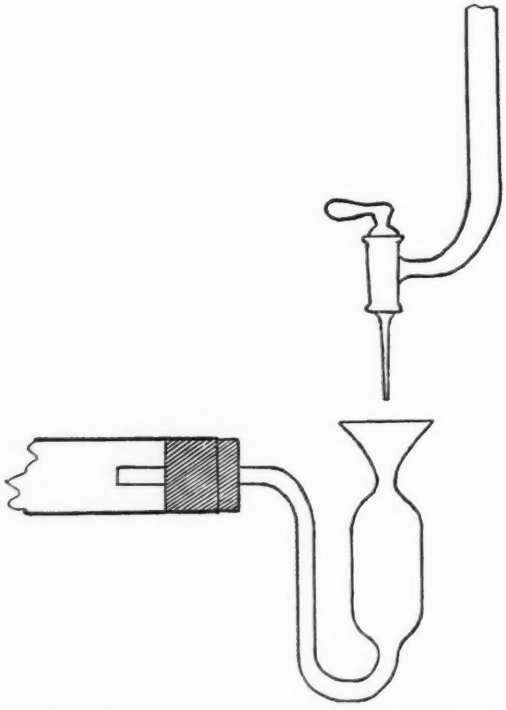

FIG. 1. FORM OF U-TUBE USED FOR REDUCING VOLUME OF LIQUID TO BE TITRATED

Cultural method

The organisms were grown in porcelain boats, so no further transfer was necessary for the estimation of the nitrogen. Four of these boats were put in a glass box the sides of which were provided with openings for two glass tubes. A current of air was passed through acid and alkali, through an aeration flask filled with water and then though the cotton-plugged glass tubes. The cultures were daily aerated during some minutes and since the air current was water-saturated, no drying out occurred. After the porcelain boats were

sterilized in the boxes, 1.5 cc. of the sterile nutrient medium was pipetted into each. One drop of a suspension of bacteria in sterile water served as inoculum. The incubation temperature was 28°C. and the period of growth was 11 to 14 days. The growth under these conditions was always very satisfactory and in the course of four days the nutrient medium of the cultures had always grown distinctly viscous.

Pea foliage extract

As this medium gave a positive reaction with diphenylamine sulfuric acid, a known, much higher amount of nitrogen was used to determine whether all nitrate nitrogen might be recovered from this medium. When to a known volume of liquid containing 0.328 mgm. of nitrogen in 1 cc., an approximately equal known volume of potassium nitrate in water (0.143 mgm. in 1 cc.) was added, the amount of nitrogen estimated appeared to correspond very closely with the amount calculated.

Estimated	0.464	0.504	0.600
Calculated	0.468	0.508	0.600

In this respect, therefore, the method of analysis proved quite safe.

In the first experiment the initial content of the medium was estimated simultaneously with the setting up of the experiment. The figures arrived at in 1.5 cc. of medium were: 0.485, 0.408, 0.482, 0.483 mgm., and the average 0.485 mgm. After 9 days the nitrogen content of the controls, having been kept under the same conditions as the cultures, was determined anew and this time 0.406 and 0.269 mgm. were found. Apparently both had lost a considerable amount of nitrogen and the checking was quite insufficient.

In the cultures inoculated with strain Pea 14, 0.503 and 0.471 mgm. of nitrogen were estimated. It will be noted that one of these cultures showed a slight increase in nitrogen, when compared with the initial content. In the early course of these experiments, when a loss of nitrogen in the controls was not yet reckoned with, the four glass boxes put up simultaneously were connected in series. In later series any nitrogen compounds set free in the porcelain boats in one of the boxes was prevented by parallel connections from passing over inoculated cultures in one of the other boxes and thus eventually being taken up.

Experiments were performed to determine whether in the absence of $CaCO_3$ such a loss of nitrogen would also occur. The absence of the $CaCO_3$ did not interfere with the growth, which, as judged by the viscosity of the medium, was quite satisfactory. The data are given in table 8. It will be noted that a loss of nitrogen occurred also in the controls. The inoculated cultures, however, when compared with the initial nitrogen content appeared not to have fixed any nitrogen (except a very slight amount in one culture where the inoculated culture had been kept side by side with the controls in the glass box). In

TABLE 8

Nitrogen content of 1.5 cc. of pea foliage extract without $CaCO_3$ inoculated by strain Pea 14

CONTROLS		CULTURES			
				Averages compared with	
Individual	Average	Individual	Average	Initial control	Control at the close
mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Initially					
0.496 0.496 0.498	0.497				
After 11 days with daily aeration					
0.429		0.487 0.437	0.462	−0.035	+0.033
After 18 days with daily aeration					
0.434		0.471 0.487 0.484	0.481	−0.016	+0.047
After 11 days without aeration (cultures and controls in one box)					
0.429		0.506 0.493	0.500	+0.003	+0.071

TABLE 9

Nitrogen content of 1.5 cc. of pea foliage extract with $CaCO_3$, inoculated by strain Pea 14

CONTROLS		CULTURES			
				Averages compared with	
Individual	Average	Individual	Average	Initial control	Control at the close
mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Initially					
0.506 0.510	0.508				
After 11 days					
0.465 0.445 0.506 0.428	0.461	0.505 0.511 0.514 0.507	0.509	+0.001	+0.048

fact the nitrogen content of the cultures had decreased, and it was only when the cultures were compared with the controls after these had been kept under

the same conditions of incubation, that a higher content of nitrogen in the cultures could be noted.

It must be pointed out here that the conclusions drawn from the earlier set of experiments in which this same comparison was made, evidently are erroneous. *The apparent increase was nothing but a smaller loss, the organism being able to use the nitrogen compounds readily set free and converting them into albuminous substances which were recovered in the analysis.* Thus the strains assumed to be *good fixers* apparently were nothing but *quick growers.*

In order to determine the difference between inoculated culture and controls an experiment was run with pea foliage extract to which $CaCO_3$ was added. In this experiment the data obtained would be directly comparable with the data of the earlier experiments. The results are given in table 9. The average

TABLE 10

Nitrogen content of 1.5 cc. of pea foliage extract, no $CaCO_3$ added, aerated preliminarily

CONTROLS		CULTURES				
					Averages compared with	
Individual	Average	Strain	Individual	Average	Initial control	Control at the close
mgm.	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*
			Initially			
0.452 0.454	0.453					
			After 13 days			
0.458 0.456	0.457	Pea 14	0.459 0.460 0.452	0.457	+0.004	
		Dalea	0.420 0.426	0.423	−0.030	−0.034

reached of 0.048 mgm., calculated on the basis of 100 cc. of medium is 3.2 mgm.

To determine whether a culture medium with stable nitrogen content could be obtained by a preliminary very thorough aeration, a strong current of air was blown through the medium, which was heated moderately. The ammonia set free was taken up in acid. This amount appeared to be very considerable, and its production extended over several hours. After this treatment the medium was sterilized and the nitrogen estimated.

In table 10 are given the results of this treatment, no $CaCO_3$ being added. Two of the strains which had been considered among the best fixers in the earlier experiments were put to the test but did not surpass the initial nitrogen. On the contrary, the Dalea strain appeared to induce a loss, which in this case, where the controls had remained stable, means a real loss.

By means of this preliminary aeration of the medium, stability in nitrogen content, when $CaCO_3$ was added, could not be attained. In several experimental series this loss in the controls varied and the conditions under which such a loss might be prevented were not worked out further.

The strains tested according to the ter Meulen method were both the working 14 and the non-working, 15, strains of the Pea organism and of the Clover organism, 205 and 202, and the Dalea strain. In table 11 the data for the pea and clover organisms are given. As the controls proved very unstable, the deviation in the behavior of the strain Pea 14 was not proved, although the

TABLE 11

Nitrogen content of 1.5 cc. of pea foliage extract with $CaCO_3$ added, aerated preliminarily

CONTROLS		CULTURES				
					Averages compared with	
Individual	Average	Strain	Individual	Average	Initial control	Control at the close
mgm.	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*
			Initially			
0.431 0.431	0.431					
			After 11 days			
0.345 0.350	0.347	Pea 14	0.388 0.376	0.382	−0.049	+0.035
		Pea 15	0.346 0.314 0.332	0.331	−0.100	−0.016
		Clover 205	0.374 0.373	0.374	−0.057	+0.043
		Clover 202	0.353 0.344	0.349	−0.082	+0.002

fact that the nitrogen content was lower than that of the controls analyzed at the close of the experiment rendered it probable that nitrogen was lost in these cultures as the result of bacterial action. In a new experiment (table 12) this assumption is confirmed. Here the loss in the controls, although not uniform, was only slight. The strains Pea 14 and Clover 205 have approximately the same slight difference from the initial controls as from the controls analyzed at the close. Strain Pea 15 however has a much lower nitrogen content than the later controls. For the Dalea strain, for which a like behavior has been reported, the same holds true here. The Clover strain 202 has lost some nitrogen as well.

From the experiments with pea foliage extract, there is no evidence of any fixation of nitrogen occurring on this substrate.

Soil extract

This medium was used with the addition of 1 per cent of glucose and $CaCO_3$. When this medium was analyzed according to the ter Meulen method, it was

TABLE 12

Nitrogen content of 1.5 cc. of pea foliage extract with $CaCO_3$ added, aerated preliminarily

CONTROLS		CULTURES				
					Averages compared with	
Individual	Average	Strain	Individual	Average	Initial control	Control at the close
mgm.	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*
		Initially				
0.325 0.326	0.326					
		After 14 days				
0.308	0.318	Pea 14	0.314	0.313	−0.013	−0.005
0.304			0.324			
0.325			0.300			
0.327						
0.329		Pea 15	0.274	0.273	−0.053	−0.045
0.317			0.274			
			0.271			
		Clover 202	0.328	0.304	−0.022	−0.014
			0.300			
			0.298			
			0.294			
			0.298			
		Clover 205	0.330	0.320	−0.006	+0.002
			0.314			
			0.308			
			0.327			
		Dalea 901	0.291	0.289	−0.037	−0.027
			0.287			

observed that after three regular checkings the amount of ammonia recovered decreased very considerably. A poisoning of the nickel catalyst was probable. This was easily explained when a high content of sulfur was detected in the medium. It was necessary, therefore, to renew the nickel catalyst after every three estimations.

TABLE 13

Nitrogen content of 1.5 cc. of soil extract

CONTROLS		CULTURES				
					Averages compared with	
Individual	Average	Strain	Individual	Average	Initial control	Control at the close
mgm.	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*
		Experiment 1, Initially				
0.056 0.054	0.055					
		After 14 days				
0.056		Pea 14	0.057 0.054 0.056	0.056	+0.001	0
		Clover 205	0.056 0.060 0.058	0.058	+0.003	+0.002
		Experiment 2, Initially				
0.074 0.076	0.075					
		After 11 days				
0.049 0.050	0.050	Pea 15	0.062 0.055 0.054	0.057	−0.018	+0.007
		Clover 202	0.065 0.053 0.050	0.056	−0.019	+0.006
		Experiment 3, Initially				
0.066 0.065	0.066					
		After 14 days				
0.050 0.051	0.051	Pea 14	0.060 0.050 0.050	0.053	−0.013	+0.002
		Pea 15	0.049 0.056	0.053	−0.13	+0.002
		Clover 205	0.063 0.058 0.049	0.057	−0.009	+0.006
		Clover 202	0.051 0.053 0.051	0.052	−0.014	+0.001

A thorough aeration was applied to the medium before use, but only a very slight amount of ammonia could be set free in this way. Only in experiment 1 (table 13) were the controls kept stable in nitrogen content: in the others a decrease was observed.

The results with soil extract are given in table 13. No increase in nitrogen can be noted in this table, when the content of the cultures is compared with the initial controls. *So here again the experiments result in no evidence for the fixation or atmospheric nitrogen.* As in the experiments with pea foliage extract, an increase in nitrogen may be noted when the cultures are compared with the controls after the period of incubation. This increase, however, is very slight, but this agrees with the fact that only a slight amount of nitrogen is lost by the controls during the course of incubation. Thus hardly any volatile nitrogenous compounds were rendered available to the organisms.

TABLE 14

Nitrogen content of yeast-water mannitol medium inoculated by strain Pea 14

CONTROLS		CULTURES			
				Averages compared with	
Individual	Average	Individual	Average	Initial control	Control at the close
mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Initially					
0.172 0.172	0.172				
After 9 days					
0.173 0.171	0.172	0.172 0.173	0.173	+0.001	+0.001

Yeast-water mannitol medium

Table 14 gives the results obtained on this medium. The uninoculated controls do not lose any nitrogen and quite in accordance with this fact, the cultures inoculated with strain Pea 14 remain on exactly the same level as the controls.

DISCUSSION

Whenever due precautions were taken against any interference with the results from loss of nitrogen from the controls, no fixation of atmospheric nitrogen could be recorded in any of the nutrient media studied. Very probably the negative results recorded in the early range of experiments with the pea foliage extract diluted down from an extract of much higher concentration and the variation in results obtained by using two batches of bean seed extract prepared individually might have been explained by a difference in the amount

of volatile nitrogenous compounds escaping from the controls. It may easily be conceived that by a longer cooking either more of the volatile substances will escape, or more albuminous substances, as the result of a more intensive breakdown of the complex proteins, will go into solution. Thus the share the volatile substances have in the total nitrogen content of the liquid will be smaller and whenever the amount of volatile substances is low, no increase of nitrogen may be noted.

The yeast-water mannitol medium, on which in the earlier experiments no fixation could be reported, when tested out along the line of the later experiments appears to lose no nitrogen during the period of incubation. This fact substantiates the assumption that the apparent fixations on the other media are to be explained by a loss of nitrogen from them. When this loss does not occur fixation will be lacking.

The amounts registered in the early range of experiments and deemed fixation, however, must still be compared in size with the amounts conceived in the later experiments, as only "apparent" fixation.

On pea foliage extract the amount for strain Pea 14 (table 13) was 2.54 mgm. average, 4.21 mgm. maximum. In table 9 the same strain gives an apparent fixation (calculated on 100 cc. of medium) of 3.2 mgm. average, 3.6 mgm. maximum. Thus these amounts are of the same magnitude.

For soil extract, however, the amounts recorded in the later range of experiments are much lower than those recorded in the earlier range. For this, two explanations are possible. Both are hypothetical, as the authentic soil extract used in the former range was not available to me. One explanation might lie in the fact that the soil extract used in the former experiments, as the result of different composition of the soil, might lose a much larger fraction of its nitrogen during the course of the experiment. In that case an apparent inconsistency in results obtained in Madison and in Delft might easily be explained. As a second explanation, it is conceivable that nitrogen occurring as nitrate in the medium might have escaped detection by the Kjeldahl method of analysis and when converted by bacterial action into albuminous substances might have been recovered by the same method (10). The soil extract used in Madison gave a reaction with diphenylamine; that used in Delft, none.

At all events these earlier experiments should not be considered as proving any fixation, for here the precaution of comparing the controls at the beginning and at the end of the experiment was omitted. For the same reason, evidence of nitrogen fixation provided by former investigators cannot be considered conclusive as long as it has not been stated distinctly that the nutrient medium was analyzed at the time the cultures were inoculated.

Whenever it has been distinctly recorded that the analysis was carried out after the incubation period, results are obviously inconclusive, and in such cases very probably the increase reported might have been explained along the same lines as the increase recorded in the earlier part of this investigation.

This is especially probable for the data of F. Löhnis, for in his experiments any of the species of bacteria tested, as far as they gave good growth, caused an increase in the nitrogen content of the medium. As the rate of growth plays an important part here, the rate being quick and only a slight amount of volatile compounds therefore having the chance to escape the explanation seems very obvious. Many investigators did not record the time of analysis of their controls, and in those cases any evidence of fixation may be deemed inconclusive.

Only very rarely are experiments recorded in which it has been stated that the analysis of the nutrient medium was carried out at the beginning and where fixation could be reported. Only such evidence could be deemed conclusive, when due notice had been taken of an initial content of nitrogen as well. Mazé fulfills this condition of analysis at the beginning, but his results could not be reproduced by later authors nor by me. Moore states that the analyses of his controls were carried out at the opening of the experiments, but as he records only an average of his data, his evidence can hardly be considered very substantial.

So in the literature, conclusive evidence is lacking for the fixation of nitrogen in pure culture by Bacterium radicicola.

On pea foliage extract a difference in the behavior of the various strains could be observed. Although the working strains Pea 14 and Clover 205 were always higher in nitrogen content than the controls analysed at the close of the experiment, strain Pea 15 appeared to be always considerably lower than these controls. The more stable the medium has kept in its nitrogen content the more distinctly this decrease in nitrogen shows (Compare table 11 and 12). So here as the result of bacterial metabolism volatile nitrogenous substances escape out of the medium.

The nitrogen content of non-working strain Clover 202 on a medium which lost much nitrogen (table 11) was lower than that of Clover 205, but not below that of the controls at the close. This behavior might be explained by a slow rate of growth. When table 12 is studied, where the results on a more stable medium are given, however, it appears that strain 202 does not reach the level of the controls. Probably this strain sets free volatile nitrogen compounds as well.

There is no reason to correlate the behavior of strains Pea 15 and Clover 202 with the inability of both these strains to supply their host plants with nitrogen, since the same phenomenon could be observed for the strain Dalea 901. This strain has been found to be an efficient supplier of nitrogen to its host plant. Since this strain had been chosen from among the strains considered as best "fixers" in the earlier experiments, a loss (table 10 and 12) induced by it was unexpected. This discrepancy can only be explained as a natural result of its being a very good grower, which shows in the very quick rate at which it induces a marked viscosity in its nutrient medium. The loss of nitrogen it induces is apparently quite masked by its quickness in taking up

the volatile nitrogen compounds set free by the medium, and the fact that by its own metabolism it sets free volatile nitrogen compounds, as well, becomes only apparent when the medium is stable in its nitrogen content.

CONCLUSIONS

1. In the media studied, *Bacterium radicicola* was unable to fix any atmospheric nitrogen.

2. It is probable that results which have been reported as positive by former investigators are to be ascribed to a loss of nitrogen from the nutrient medium.

3. All evidence available points to the inability of *Bacterium radicicola* to fix nitrogen in the absence of the host plant.

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DEHYDRATION AND SOIL ACIDITY

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It has long been a matter of common knowledge that the removal of water from the soil, such as occurs naturally in summer in this climate and more completely in regions of the earth with higher summer temperatures, produces far-reaching changes in some of the more important soil constituents, and exerts a considerable influence upon their fertility as measured by the crop which these soils are capable of producing. It has generally been believed that summer sun and the baking that land receives in warmer latitudes have a valuable effect in making the land more productive and setting free some of the reserves of plant-food. The benefit that any crop will receive from these changes will depend on the extent to which this action is reversible and also on the rate at which this reverse action takes place.

There is a considerable volume of literature of recent years dealing with various aspects of the subject, but as much of this is contradictory and deals with widely different problems, it seemed to us desirable to attempt to obtain further information upon one very important aspect of this question, in the light of recent work on soil constitution. As will appear from a perusal of this paper, the results which we have so far been able to obtain indicate sufficiently that the importance of the work is such that no view of soil fertility, no conception of the relation between soil and plant growth is complete which does not take into account the nature and extent of these changes. It appears to us that work of this kind suggests that even some of the most valued soil constants are rather less constant than had been imagined, and that it is necessary to substitute for a single value in respect to one property, a range of possible values depending upon the conditions under which they are determined.

So far it has been possible for us to deal fully with only one of the soil properties exhibiting this range. An example may be quoted to illustrate the meaning of soil range in relation to one particular property. It is usual to state that the hydrogen-ion concentration of a soil-water suspension is given by a pH value of 5, but it appears that it is not possible to express this property in such a definite way, as the value obtained experimentally will depend on the degree of hydration of the soil when suspended in water, and therefore a range of values from that given by a suspension of soil in its natural moist condition to that given by a suspension of the same soil after dehydration under definite conditions must be substituted for the single values.

The following review of the more important literature of the subject gives some idea of the scope of the work which has been carried out so far.

A complete bibliography of the work previous to 1922 has been given by Gustavson (7).

Burgess (4) worked with Miami silt loams. He found that air-and oven-drying (at 103°C.) had little effect on acid soils, but made alkaline soils somewhat less alkaline (a change of 0.4). He suggested that the effects might be due to oxidation, or exposure of new surfaces by removal of the colloidal covering.

Rost and Fieger (10), found that air-drying generally increased the hydrogen-ion concentration in both acid and alkaline soils, and oven-drying increased it still further. Samples moistened after drying became more acid than the original moist samples and often more acid than the air-dried samples.

Baver (2) said that air-drying caused no significant change in pH value, but that grinding the soil produced varying changes. He found that the amount of lime present affected the pH change on heating, an increase in the lime causing a decrease in the change. With subsoils he obtained a marked increase in acidity on air-drying, the effect increasing with depth. He suggested that the increase of acidity from May to September was possibly due either to dehydration of colloids and consequent decrease of buffer action, or to accumulation of soluble matter.

Joseph and Martin (8) found that a high temperature and desiccation of alkaline soils reduced the alkalinity. The pH was measured electrometrically in 1:5 suspensions. They stated that the effect was probably due to the accelerated effect of the electrolytes caused by increase of temperature and concentration, since the same value was obtained for dried and undried samples if both were left in contact with water for one week.

Krickmann (9) said that exchange and hydrolytic acidity were relatively little altered by drying. Active acidity was twice as great after a week's drying and four times as great after six months. Above 100°C. the acidity was forty times as great. He said that as the increase was proportional to the humus content, it was the organic matter that caused the results. The active acidity decreased at temperatures above 150°C. and vanished at red heat. The exchange and hydrolytic acidity resisted change because of their partially inorganic origin.

Achromeiko (1) said that sun-drying made the soil slightly more acid, and drying at higher temperatures further increased the acidity. After being wet and stored, the pH of the dried soil again approached the neutral point. This change was set down to the effect of micro-organisms, as under sterile conditions no such changes were observed. The increase of acidity at higher temperatures was due to the increase of acid organic compounds. The solubility of organic matter was very little changed by storing dried soils in a damp sterile condition, but the solubility of phosphoric acid was greatly reduced because of the adsorption of phosphate by the soil. In drying and heating, the organic part of the disperse phase was considerably increased, whereas the mineral part was almost as much decreased. Drying in a thermostat affected the mineral and organic matter equally, whereas sun-drying had more effect on the mineral part of the disperse phase. When the dried soil was stored in a moist condition the organic part of the disperse phase was diminished while the mineral part was increased. Hence the soil came back to a state similar to the original damp soil.

Bülman (3) said that air-drying did not significantly change the pH.

Ellis and Morison (6) found that the removal of water from peat caused a relative increase of water-extractable ammonia, because of a reduction in the adsorptive properties of the peat. A partial recovery in the adsorptive power took place on prolonged treatment with water, equilibrium being reached in 10 days.

Steenkamp (12) found that cultivated soils on dehydration showed an increase in the amounts of exchangeable bases and in the degree of saturation, the increase being greatest for soils containing humus. Dehydration of pasture soils, however, gave opposite results. He stated that air-dried soil of high humus content showed reversibility of the colloidal condition after 21 days.

Soubrahmanyan (11) reported a production of ammonia in water-logged soils, and a consequent rise in pH. This was probably due to enzyme action and not to any biological factor.

The general results of the earlier investigators in this subject point to the fact that the drying of the soil considerably increases the amount of water-soluble substances, both organic and inorganic. This increase in water-soluble substance was associated with changes in biological activity, and with an increase in the solubility of the organic matter.

The effect of heat upon the bacterial flora, and the interesting changes following partial sterilization, to some extent have obscured the less obvious but no less important change produced in the inorganic soil colloids, and in the soil reaction. Most of the later investigators are agreed that dehydration by heat, and to a smaller extent dehydration at low temperatures, causes an increase in acidity.

The recovery of air-dried soils when in contact with water does not appear to be definitely established, though it would seem that in some cases a partial reversion to the original state occurs.

The main object of this communication is therefore to produce conclusive evidence on one aspect of the dehydration problem, namely, changes in acidity.

EXPERIMENTAL METHODS

The method employed in measuring the pH of the soils was that described by Bülman (3). Table 1 gives a list of the soils employed, together with their chemical analyses, which were worked out under the new international method.

The object of the first set of experiments was to investigate the effect on the pH of drying and heating the soils in a steam oven at 98°C. The pH was measured in water suspension and also in normal potassium chloride suspension, and the percentage of water in the soils for each measurement was determined. The soil: water ratio was 1:2 by volume in every case. The results obtained are given in table 9, and the water suspension values are illustrated in figures 1, 2, and 3, where the pH values are plotted against the time of heating.

It will be seen that in every case the soils showed a definite increase in acidity after prolonged heating. They showed also that in the case of all the soils examined except the peat, the greatest changes took place in the first two days, that is, while the water was being driven off. After that the reaction became more acid until the pH became approximately constant after 10 or 12 days' treatment in the oven. The peat was an exception to this, no appreciable change in the pH being evident until the water had been driven off, after which the peat gradually became more acid, reaching a constant figure after 11 days.

A further set of experiments was carried out in which the acidity was determined in a normal solution of potassium chloride instead of water. The results in this case were similar, though showing a higher acidity throughout. On continued drying, the increase in acidity produced by potassium chloride decreases, and, in the case of the soil containing calcium carbonate, vanishes.

The next set of experiments was made with the object ot determining the effect on the pH of drying the soils in a desiccator at room temperature. Soils 3, 4, 6, 7, 8, and 9 were dried for 14 days over sulfuric acid, and soils 10 and 11 were dried for 28 days. Some of the soils after the desiccator drying were heated for 24 hours in the steam oven. Table 2 shows the results obtained. It is clear from these figures that drying the soils at ordinary temperatures did not produce as much change in the pH as did drying them in an oven. The results obtained with the peat (soil 10) are interesting, as they confirm the results obtained by oven-drying the peat, which indicated that the change in pH did not begin until the water had been driven off. Again it is noticeable that the four soils which were afterwards heated for 24 hours in the oven showed a much greater change in pH after this heating than they did after the 14 days' desiccator drying.

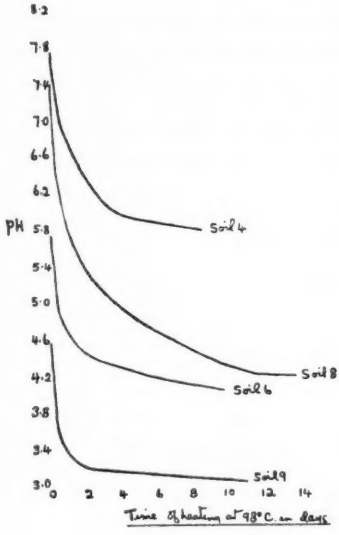
TABLE 1

Mechanical analysis of soils recorded on the basis of dry soil

ORIGIN AND DESCRIPTION OF SOIL	NUMBER	LOSS ON IGNITION	COARSE SAND	FINE SAND	SILT	CLAY	CALCIUM CARBONATE
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Calcareous grit. Sandy humus from forest	3	12.26	57.90	18.65	7.94	3.11	....
Coral Rag:							
An arable clay soil	4	7.24	22.96	16.52	14.17	33.02	4.61
Kimeridge clay:							
A heavy silt loam pasture soil	6	7.24	23.47	26.53	19.87	24.01	....
A light clay pasture soil	7	10.67	13.67	20.94	22.16	33.42	....
A heavy clay pasture soil	8	14.25	2.62	6.20	17.54	60.72	....
A forest subsoil clay soil	9	4.78	17.33	22.34	19.05	34.81	....
Northampton sand. An arable soil	11	4.64	66.31	13.27	4.73	9.28	....
Peat from the west of Ireland	10	.....	.....	.....	.....	.....	....

The next point to be investigated was whether these changes in the pH were permanent or whether the soils tended to become less acid on prolonged contact with water.

For this purpose the soils were dried in the steam oven and then were put into a number of test tubes, each tube containing the soil and water in the same ratio as was used for the previous pH determinations, and shaken from time to time. The pH of the soil suspensions was taken at intervals up to two months, only one measurement being made with each suspension, so as to avoid any effects the quinhydrone might produce by prolonged contact with the soils. The results are given in table 3. The figures showed that in the peat (soil 10) there was no recovery at all, and that in the sandy humus soil (soil 3) and the sand (soil 11) the recovery was very slight. In all the other soils, which contained considerably more clay than soils 3 and 11, there was a very significant recovery, and in the four heaviest soils (soils 4, 7, 8, and 9) the pH after two



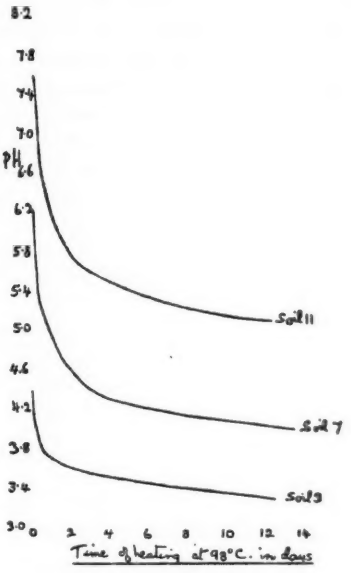


FIG. 1 FIG. 2

FIG. 1. EFFECT OF HEATING UPON THE ACIDITY OF SOILS 4, 6, 8, AND 9
FIG. 2. EFFECT OF HEATING UPON THE ACIDITY OF SOILS 3, 7, AND 11

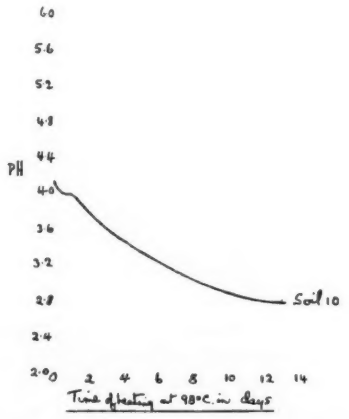


FIG. 3. EFFECT OF HEATING UPON PEAT

months rose very nearly to that of the original moist soils. This seemed to afford definite evidence that it was the mineral colloidal matter, and not the organic colloidal matter, which caused recovery from the heating effects, and suggested that any explanation involving the biological production of ammonia in the water-logged soils was unlikely, as in that case the organic soils should have recovered as much as, or more than, the mineral ones.

It was then decided to try to discover whether the increase of acidity produced by heating was caused by some change in the physico-chemically ab-

TABLE 2

Effect of desiccator drying on the soils

SOIL NUMBER	BEFORE DRYING		AFTER DRYING		AFTER 24 HOURS IN OVEN	
	pH	Per cent H_2O	pH	Per cent H_2O	pH	Per cent H_2O
3	4.38	20.31	3.98	2.30	....	....
4	8.01	.....	7.11	....	....	....
6	5.74	16.31	5.45	0.54	4.39	0
7	6.22	24.38	5.92	1.02	4.72	0
8	7.40	23.95	6.79	1.10	5.50	0
9	4.57	23.82	4.43	1.23	3.50	0
10	4.12	88.20	4.12	0	....	....
11	7.58	15.59	6.87	0	....	....

TABLE 3

Recovery experiments on the dried soils

SOIL NUMBER	SOIL REACTION AFTER										
	0 day	1 day	3 days	6 days	10 days	15 days	21 days	28 days	42 days	56 days	Wet
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
3	3.63	3.57	3.60	3.67	3.66	3.73	3.77	3.83	....	3.90	4.38
4	6.25	6.62	7.05	7.33	7.55	7.73	7.84	7.85	....	7.89	8.01
6	4.30	4.49	4.52	4.55	4.71	4.73	4.79	4.80	5.16	5.06	5.74
7	4.97	5.03	5.18	5.33	5.33	5.31	6.05	5.95	6.07	6.13	6.22
8	5.97	5.98	6.06	6.37	6.74	6.89	7.10	6.95	....	7.26	7.40
9	3.48	3.67	3.77	3.78	3.80	3.92	3.98	4.00	4.09	4.35	4.57
10	3.05	3.08	3.06	3.04	3.05	3.04	3.07	3.05	....	3.04	4.12
11	5.71	5.98	5.88	6.07	6.07	6.27	6.26	6.33	....	6.32	7.58

sorbed bases (the exchangeable bases of Hissink) or whether it was due to the release of acid substances physically adsorbed by the soil colloids. For this purpose the bases were removed from the soils by means of dilute hydrochloric acid, and after being washed till free from chloride, the soils were dried in the steam oven in the usual way. The experimental details were obtained from Comber (5).

The figures obtained are given in table 4 and showed that with the excep-

tion of the sandy humus soil, 3, the pH was not appreciably changed by drying these soils. It is very significant that in all cases approximately the same figure was obtained, indicating that the acidity was independent of the amount of clay present, and that probably the same substance was the cause of the acidity in every case.

TABLE 4

Effect of oven-drying on the pH of base-free soils

SOIL NUMBER	REACTION AFTER						
	0 day	0.25 day	0.5 day	1 day	2 days	3 days	4 days
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
3	2.96	....	2.80	2.68	....	....	2.53
4	2.96	....	....	2.98	....	2.89	....
6	2.86	3.01	....	2.86	....	2.82	....
7	2.92	....	....	2.86	....	2.75	....
8	3.05	....	2.83	2.84	....	....	2.69
9	2.82	....	3.09	2.99	....	....	2.85
11	3.10	3.11	....	2.86	....	2.86	....

TABLE 5

Effect of oven-drying on the pH of the soils containing individual cations

SOIL	REACTION AFTER									
	0 day	0.25 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
Calcium soil 6	3.95	3.45	3.29	3.11	....	2.97	....	....	....	....
Barium soil 11	8.59	....	7.47	6.81	....	6.23	....	5.78	....	5.64
Iron soil 8	2.86	....	2.60	....	2.48	....	2.43	....	2.47	....
Iron soil 9	3.24	....	2.75	....	2.65	....	2.55	....	....	....
Ammonium soil 4	8.06	....	4.46	4.07	....	3.86	....	3.65	....	3.64
Sodium soil 4	7.55	....	5.09	4.40	....	4.16	....	....	....	....
Sodium soil 9	8.93	....	6.47	5.07	....	4.73	....	4.40	....	....
Saturated potassium soil 7*	10.5	....	8.5	....	....	8.00	....	....	....	....
Saturated potassium soil 11*	10.5	....	....	....	....	8.5	....	....	....	....

* pH values measured by the B. D. H. Capillator.

These moist base-free soils were then treated with solutions of various metallic cations in order to produce soils containing considerable quantities of individual metallic cations in an exchangeable form. In this way soil 6 was treated with normal calcium chloride to make a calcium soil; soils 8 and 9 were treated with normal ferric chloride to make iron soils; soil 11 was treated with fifth normal baryta to make a barium soil; soils 4 and 6 were treated with normal sodium carbonate to make sodium soils; soil 4 was treated with ammonium hydroxide to make an ammonium soil; and soils 7 and 11 were treated with normal potash to make saturated potassium soils.

The excess of the various reagents was removed by washing. In some of the cases where alkalies were used, some of the organic matter was dissolved, which made filtering impossible. These soils therefore had to be washed by decantation in the course of which some of the fine clay was lost. The filtering of the barium soil was done in an atmosphere free from carbon dioxide.

The results of heating these soils are given in table 5. These results show very clearly that it was the exchangeable bases that were responsible for the increase of acidity on drying, as all the soils showed increase of acidity, and the two sodium and the ammonium soils showed a very remarkable increase. Also it was significant that the saturated soils showed a considerable increase

TABLE 6

Effect of heat on base-free inorganic soils

SOIL NUMBER	REACTION AFTER HEATING FOR			
	0 day	1 day	3 days	6 days
	pH	*pH*	*pH*	*pH*
6	2.58	3.05	2.96	2.81
8	2.56	2.99	2.87	2.80

TABLE 7

Effect of heat on the total exchangeable bases

(Mgm. equivalents per 100 gm. dry soil)

SOIL NUMBER	EXCHANGEABLE BASES	
	Before heating	After heating
3	4.37	3.22
6	9.25	7.37
7	19.94	16.56
8	39.73	31.36
9	4.00	1.26
11	12.70	11.24

of acidity on drying. These soils differed from those in table 4 only in having some of the hydrogen replaced by metallic cations.

As an additional experiment two soils, 6 and 8 were treated with hydrogen peroxide and hydrochloric acid, as in mechanical analysis, to remove organic matter and exchangeable bases, and then washed free from acid. On being oven-dried these soils showed no appreciable change of pH (table 6).

These experiments show the result of heat and dehydration upon the acidity of soil suspensions.

The next series of results are an attempt to ascertain the direction in which the other constituents of the soil complex are affected.

In the cultivated soils on which Steenkamp worked the total exchangeable

bases and the degree of saturation of the soils were increased on drying and such soils should show a corresponding decrease in acidity. As all the soils (both cultivated and pasture) in the present work behaved in the reverse manner, it was decided to find out whether there was any increase or decrease in the exchangeable bases on drying in the steam oven. The experimental details were those described by Steenkamp (12), determinations being made on the fresh soils, and on those that had been treated for 48 hours in the steam oven. In every case it was found that drying produced a decrease in the total exchangeable bases (table 7).

Estimations were also made of the amounts of phosphate, calcium, and, in one case, potassium which could be dissolved out by water both before and after heating.

In each case 100 gm. of soil was shaken mechanically for one hour with one liter of distilled water. In some cases the dried soils were left for 15 days, after

TABLE 8

Effect of heat on the amounts of substances extractable by water

(Mgm. per 100 gm. soil)

SOIL	PHOSPHATE (P_2O_5)			CALCIUM			POTASSIUM		
	A	B	C	A	B	C	A	B	C
3	1.88	4.84	7.54	2.91	7.08	10.45	....	....	....
6	1.51	2.54	....	13.97	29.70	.....	....	....	....
7	1.70	2.49	1.97	9.92	37.31	35.13	4.47	6.28	3.74
8	2.44	4.14	....	14.20	54.40	.....	....	....	....
11	3.76	5.94	....	9.86	23.45	.....	....	....	....

A—Fresh soil.

B—Soil dried for 48 hours in the oven.

C—Dried soil left for 15 days in contact with water.

the mechanical shaking, in contact with water with occasional handshaking to see whether any of the compounds were readsorbed. The results, expressed as milligrams of compound per 100 gm. of dry soil, are given in table 8.

Calcium showed the largest increase in all cases. The sandy humus soils did not show any readsorption of phosphate and calcium on prolonged contact with water, but the light clay soil, 7, showed a considerable readsorption of phosphate and potassium but only a slight readsorption of calcium.

At this stage in the investigation it is not proposed to attempt to give an explanation of these results or to put forward a view of the constitution of the soil particles which is in agreement with the results.

The experimental work has demonstrated beyond question the following facts:

1. All mineral soils showed a considerable decrease of pH on the removal of such water as can be driven off at 98°C., followed by a slight decrease on further heating.

TABLE 9

Effects of heat on the pH of soils

Soil 3

	TIME OF HEATING								
	0 day	0.46 day	1 day	2 days	4 days	6 days	8 days	10 days	12 days
Per cent water	20.31	0.26	0	....	....	....	....	....	....
pH in water	4.38	3.74	3.69	3.71	3.53	3.42	3.39	3.33	3.30
pH in *N* KCl	3.38	3.21	3.18	3.16	3.08	2.97	2.92	2.88	2.88
Difference	1.00	0.53	0.51	0.55	0.45	0.45	0.47	0.45	0.42

Soil 4

	TIME OF HEATING										
	0 day	0.25 day	0.5 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days
Per cent water	5.94	0.21	0.05	0	....	....	....	....	....	....	....
pH in water	7.76	7.23	6.98	6.76	6.36	6.07	5.96	5.92	5.89	5.82	5.82
pH in *N* KCl	6.97	6.56	6.43	6.31	6.08	5.90	5.89	5.88	5.88	5.82	5.82
Difference	0.79	0.67	0.55	0.45	0.28	0.17	0.07	0.04	0.01	0	0

Soil 6

	TIME OF HEATING										
	0 day	0.21 day	0.46 day	1 day	2 days	3 days	5 days	7 days	9 days	11 days	13 days
Per cent water	16.31	0.74	0.71	0.30	0	....	....	....	....	....	....
pH in water	5.74	4.99	4.82	4.63	4.38	4.38	4.26	4.12	4.07	3.84	3.67
pH in *N* KCl	5.00	4.59	4.26	4.19	3.96	3.92	3.73	3.69	3.64	3.47	3.28
Difference	0.74	0.40	0.56	0.44	0.42	0.46	0.53	0.43	0.43	0.37	0.39

Soil 7

	TIME OF HEATING										
	0 day	0.21 day	0.46 day	1 day	2 days	3 days	5 days	7 days	9 days	11 days	13 days
Per cent water	24.38	0.49	0.04	0	....	....	....	....	....	....	....
pH in water	6.22	5.35	5.10	4.97	4.76	4.36	4.28	4.14	4.15	3.99	4.00
pH in *N* KCl	5.60	4.86	4.69	4.52	4.30	3.99	3.88	3.75	3.73	3.61	3.62
Difference	0.62	0.49	0.41	0.45	0.46	0.37	0.40	0.39	0.42	0.38	0.38

TABLE 9—*Concluded*

Soil 8

	TIME OF HEATING										
	0 day	0.21 day	0.46 day	1 day	2 days	3 days	5 days	7 days	9 days	11 days	13 days
Per cent water	23.70	0.15	0.07	0	....	....	....	....	....	....	....
pH in water	7.40	6.44	6.24	5.79	5.34	5.09	4.76	4.63	4.38	4.19	4.22
pH in *N* KCl	6.28	5.95	5.78	5.40	4.95	4.70	4.48	4.34	4.06	3.87	3.93
Difference	1.12	0.49	0.46	0.39	0.39	0.39	0.28	0.29	0.32	0.32	0.29

Soil 9

	TIME OF HEATING								
	0 day	0.21 day	0.46 day	1 day	2 days	4 days	6 days	8 days	10 days
Per cent water	23.82	....	0.37	0	....	....	....	....	....
pH in water	4.57	3.66	3.57	3.33	3.18	3.18	3.12	3.07	3.07
pH in *N* KCl	3.71	2.86	2.78	2.62	2.61	2.54	2.57	2.59	2.58
Difference	0.86	0.80	0.79	0.71	0.57	0.64	0.55	0.48	0.49

Soil 10

	TIME OF HEATING									
	0 day	0.21 day	0.42 day	1 day	3 days	5 days	7 days	9 days	11 days	13 days
Per cent water	88.20	36.38	0.12	0.05	0	....	....	....	....	....
pH in water	4.12	4.04	4.00	3.99	3.49	3.38	3.11	2.98	2.80	2.81
pH in *N* KCl	2.99	3.03	3.27	2.99	2.75	2.66	2.58	2.46	2.40	2.38
Difference	1.13	1.01	0.73	1.00	0.74	0.72	0.53	0.52	0.40	0.43

Soil 11

	TIME OF HEATING									
	0 day	0.25 day	0.42 day	1 day	2 days	4 days	6 days	8 days	10 days	12 days
Per cent water	15.59	0	....	....	....	....	....	....	....	....
pH in water	7.58	6.79	6.54	6.13	5.74	5.47	5.33	5.21	5.14	5.08
pH in *N* KCl	6.76	6.29	6.20	5.85	5.54	5.31	5.20	4.99	4.95	4.81
Difference	0.82	0.50	0.34	0.28	0.20	0.16	0.13	0.22	0.19	0.27

2. The peat showed no change in pH until all the water had been removed, and then only a slow change.

3. The changes produced in the mineral soils were reversible, whereas those produced in the organic ones were not.

4. The two soils which were fully saturated with bases showed a decrease in pH on drying.

5. The change in pH produced by heating the soils from which the exchangeable bases had all been removed by dilute hydrochloric acid was insignificant.

6. The soils produced by treating the base-free soils with various metallic cation solutions—in order to supply them with exchangeable bases—all showed a considerable decrease in pH after being dried at 98°C.

7. After being dried, the soils contained a smaller quantity of exchangeable bases than the original moist soils.

8. After being dried, the soils showed increases in water-soluble calcium, potash, and phosphate.

9. The magnitude of the increase in acidity depended on the original pH rather than on the amount of clay present, the alkaline soils showing greater changes than the more acid ones.

10. The increase in acidity was caused by some change in the physico-chemically absorbed bases.

The alternative to this was that partial dehydration of the soil colloids released some of the physically adsorbed compounds, and if these were of an acid nature they could cause the observed results. In the latter case the removal of the exchangeable bases by dilute hydrochloric acid should not effect the increase in acidity on drying. To test this all the soils were thus treated, and the results given in table 4 showed that drying did not materially affect the pH. This suggested that the physically adsorbed compounds were not responsible. It was conceivable, however, that these compounds had been largely washed out in the course of washing the soils free from chloride. But in this case the pH should not change on drying if some exchangeable bases were put into the soil. Table 5 showed conclusively that soils so treated changed considerably on drying, the pH in every case falling. We concluded, therefore, that the exchangeable bases must in some way be responsible for the increase in acidity caused by drying.

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THE EFFECT OF PHOSPHATE AND LIME UPON THE RATE CURVE OF SOLUBILITY OF PHOSPHORUS FROM A WOOSTER SILT LOAM SOIL[1]

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The study of the phosphate ion through the analysis of a soil extract has led to divergent views concerning the behavior of phosphate in the soil system. An intimate knowledge of how the reactive bases of the soil have combined with its natural phosphates would indicate whether phosphate added as a fertilizer would be precipitated as an insoluble compound or retained in a more soluble form. It was suggested twenty years ago by Whitson and Stoddard (10) that acid soils act as if deficient in phosphorus because of the formation of aluminum and iron salts. Recently Vanstone (9) has given confirmatory evidence of the relative solubility of phosphorus of acid soils in his experiments upon the solubility of soils in citric acid. Soils of acid reaction tend to form one group by this scheme of extraction, and those soils containing calcium carbonate or of alkaline reaction, another, depending upon the solubility of phosphates.

In a summary of a recent investigation upon the concentration of phosphorus in the soil solution from water extracts and displacement methods, Parker (6) concludes that, since the displaced solution of many productive soils contains a trace of inorganic phosphorus, it seems necessary to assume that plants do not obtain all their phosphorus from the soil solution indicated by a displacement method. Apparently the solid phase of the soil has an important function in phosphorus nutrition of plants. If there were no renewal of phosphate in a soil solution that contains 1 to 2 p.p.m., the crop would need impossibly large amounts of solution to furnish the 25 to 70 pounds of phosphate required. The rapidity of this renewal is indicated by the fact that a 1:5 water extract, though aiming at nothing more than the dilution of the soil solution, does bring out much more phosphate. In referring to this low concentration of phosphate in the soil solution, Teakle (9) concludes that to calculate this concentration to a dry soil basis would given an erroneous impression. Russell and Prescott (7) explain that this low concentration is due mainly to adsorption, and conclude that their results of fixation of phosphates in the soil may be expressed by the Freundlich Equation.

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Russell and Prescott observed that two separate actions occurred simultaneously during the period of extraction of a soil—as the phosphate dissolved rapidly in the acid, it reacted with some constituent and was reprecipitated. Thus the dissolving action of all mineral acids of equivalent concentration was the same, but the "reverse action" differed, hence the net amount of phosphate determined by the analyst was different. This reverse action was regarded as adsorption by Russell and Prescott, but Fisher (3) and Comber (1) have both shown that it is more of the nature of a chemical precipitation. Comber made the contribution that this precipitation is possible in the presence of a mineral acid, for the acid acts in two ways, i.e., more calcium phosphate is being dissolved as well as more iron and aluminum, which precipitate the phosphate from the extract. Very little precipitation occurs when citric acid is used, for the iron and aluminum phosphates remain in solution, thus the phosphate content of the extract does not become lower as it does when mineral acids are used.

A 1 per cent citric acid has been used by Dyer (2) to measure the response of soils to phosphatic manuring. The limit of .01 per cent phosphorus pentoxide was used to designate soils that respond to phosphate treatment, but later Hall, Wood, and Berry (4) showed that this limit was too low for pasture soils. In placing the limit from .01 per cent to .02 per cent phosphorus pentoxide, which agreed better for pasture responses, more citric acid was necessary for the conditions of the method because complete neutralization often had occurred. In a summary it is agreed that field trials correlate better with the 1 per cent strength of acid. Lemmermann (5) used the term "the relative solubility" to differentiate soils that did from soils that did not respond to phosphatic treatment. When this ratio of soluble to total phosphorus reached a value of 40 to 60 per cent a soil did not respond to treatment, but those of 25 per cent or less did respond. Andre and Copaux conclude from experiments based on farm practice, that it is not so much the absolute dose of phosphate as the size of this ratio that measures the factor of fertility.

In a recent investigation, Vanstone (10) by making a series of extractions of increasing weights of soil, has elaborated upon the method of Dyers' single extraction. By using the milligram of phosphorus soluble in an extract of 20, 40, 60, and 80 gm. of soil as the abscissa and the total phosphorus expressed in grams in each of these weights of soil, respectively, as the ordinate, a rate curve of solubility is developed. (The curve is called a "rate curve" because it indicates the amount of phosphorus dissolved for a given time.) The curve for a soil that has its phosphorus largely combined as iron and aluminum will approach the ordinate axis. The rate curves for soils containing increasing amounts of calcium will approach the abscissa or the curve for complete solubility of calcium phosphate. Thus, Vanstone found that the rate curves tend to group soils of acid reaction near the ordinate and those of alkaline properties near the abscissa.

EXPERIMENTAL

In order to determine the difference in the amounts of phosphorus due to reprecipitation or adsorption during the extraction, equal weights of an uncropped soil were extracted for one hour by 1 liter of tenth normal sulfuric, nitric, and citric acids. This soil is a Wooster silt loam and lies adjacent to plots whose crop response to phosphate manuring is known. The soil was air-dried and prepared to pass a 2-mm. sieve. The soluble phosphorus extracted by each acid is given in table 1.

The data in table 1 indicate that the soluble phosphorus depends upon the total amount present. The ratios of soluble to the total phosphorus diminish rapidly as the amount of total phosphorus increases. The ratio of soluble to total phosphorus for citric acid decreases from 16.8 to 9.0, a greater decrease of 15.6 to 6.6 is noted for sulfuric acid, and a still greater decrease to 3.7 appears for nitric acid. As Russell and Prescott maintain that the solvent action for all

TABLE 1

The soluble phosphorus from tenth normal sulfuric, nitric, and citric acids and the ratio of soluble to total phosphorus

WEIGHT TAKEN	TOTAL P PRESENT	P SOLUBLE IN 0.1 *N* ACID			$100 \times \frac{\text{SOLUBLE P}}{\text{TOTAL P}}$		
		H_2SO_4	HNO_3	Citric	H_2SO_4	HNO_3	Citric
gm.	*gm.*	*gm.*	*gm.*	*gm.*			
20	0.0083	0.0013	0.0010	0.0014	15.6	12.0	16.8
40	0.0166	0.0019	0.0013	0.0026	11.4	7.8	15.6
80	0.0332	0.0027	0.0016	0.0041	8.1	4.8	12.0
160	0.0664	0.0044	0.0025	0.0061	6.6	3.7	9.0

acids is the same, a reverse action has reduced the amount of soluble phosphorus in the extracts of all weights of soil. The greatest difference (due to reprecipitation or adsorption) appears when 160 gm. of soil is extracted, for only 41 per cent of the phosphorus for nitric acid and 73 per cent for sulfuric acid remain in the extract when compared to the quantity remaining in the citric acid extract. It appears that iron and aluminum pnosphate may be precipitated in the presence of free mineral acid, as Comber claims, and also that the former phosphates are more soluble in citric acid than in mineral acids.

According to the limits established by Lemmermann, this soil should respond to phosphatic manuring regardless of the choice of acids used for extraction. In order to test the effect of cropping and fertilizer treatment upon the rate curve of solubility for phosphorus in this soil, two tracts of extreme treatment were used as sources of soil to represent these conditions. For a soil which should show an apparent reduction in soluble phosphorus, an unfertilized plot that had been continuously cropped to corn for a long period was used. As the source of a soil which is in a high state of fertility, the variety

test field—a four-year rotation of corn, oats, wheat, and clover, receiving liberal applications of phosphate, manure, and lime was selected.

The soluble phosphorus from 0.1 *N*-extracts of Wooster silt loam under two systems of culture is compared with the original uncropped soil in table 2.

It is significant that the two widely different treatments of this soil for a long period have had a marked effect upon the soluble phosphorus remaining above that required by the crops. Continuous cropping has slightly depleted the soluble phosphorus below that the uncultivated soil, whereas the liberal applications of phosphate with additional lime have increased greatly the soluble phosphorus in the soil from the the Four-Year Rotation or Variety Test Tract. The effect of the treatments upon the rate curve of solubility is shown in figure 1.

TABLE 2

The soluble phosphorus from tenth normal sulfuric acid extractions of Wooster soil under two extreme systems of treatment

SOIL	WEIGHT	PHOSPHORUS PRESENT	SOLUBLE P	$\frac{100 \times \text{SOLUBLE P}}{\text{TOTAL P}}$
	gm.	*gm.*	*gm.*	
Uncultivated and uncropped, total phosphorus 820 pounds to the acre	20	0.0083	0.0013	15.6
	40	0.0166	0.0019	11.4
	80	0.0332	0.0027	8.1
	160	0.0664	0.0044	6.6
Continuous culture corn unfertilized, total phosphorus 810 pounds to the acre	20	0.0081	0.00126	15.5
	40	0.0162	0.00190	11.1
	80	0.0324	0.00255	7.8
	160	0.0648	0.00320	4.9
	320	0.1296	0.00370	2.8
Variety field test 4-year rotation, total phosphorus 1,224 pounds to the acre	20	0.01224	0.0037	30.2
	40	0.02448	0.0057	23.2
	80	0.04896	0.0098	20.0
	160	0.09792	0.0156	15.9
	320	0.19584	0.0239	11.6

The rate curves for an unfertilized soil continuously cropped to corn are compared to one treated liberally with phosphate, manure, and lime.

It is evident that the phosphorus is very effective in producing crop responses in the scheme of fertilizing used or the Variety Text Tract, and it is retained in a combination quite soluble to tenth normal sulfuric acid. The rate curve of solubility slopes toward the abscissa, approaching the curve for complete solubility of calcium phosphate. The position of the curve places this soil in the region occupied by soils of alkaline reaction, which is verified by the pH of 7.1 for this soil. The ratios from the extracts of the Variety Field have increased 100 per cent over those from the uncropped soil, which is a greater percentage

increase than that of the total number of pounds of phosphorus an acre. This condition appears to indicate that the phosphorus is retained in combination with calcium and not in the more insoluble forms of iron and aluminum. The rate curve for the soil subjected to continuous cropping without treatment appears slightly closer to the ordinate than the curve for the original uncropped soil. This change appears to be due to the combination of the phosphorus rather than to the removal by crops, in this soil with a pH of 5.

Since the "rate curves" show a significant difference in the combination of phosphate in this soil under widely different treatments, the question of how lime alone would affect a smaller application of superphosphate in a longer

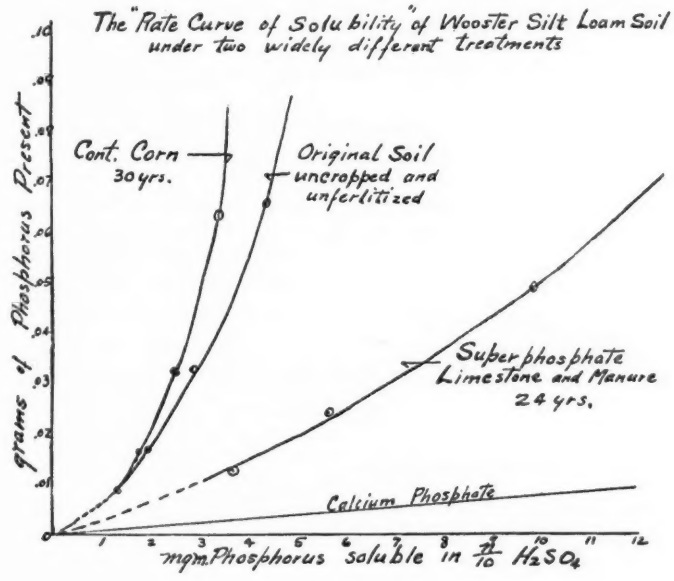

FIG. 1

rotation was investigated. A soil approaching this condition is that of plot 2, section D, 5-year rotation, which receives 320 pounds superphosphate a rotation and is limed on one end. The soluble phosphorus from the extractions of this soil appears in table 3 with an unlimed check plot for comparison.

The phosphorus in the soil from the limed end of plot 2 appears to remain in a more soluble combination and likewise the ratios of soluble to total phosphorus are significantly higher. This difference in solubility, although smaller than those found in table 2, which is a result from soils of widely different treatments, appears to be solely due to the lime, as one would expect very little organic phosphorus to be extracted from the crop residues.

TABLE 3

The soluble phosphorus from tenth normal sulfuric acid extractions of a limed and unlimed phosphated soil, compared to an unfertilized plot

SOIL	WEIGHT	PHOSPHORUS PRESENT	SOLUBLE P	$\frac{100 \times \text{SOLUBLE P}}{\text{TOTAL P}}$
	gm.	gm.	gm.	
Plot 2. 5-year rotation limed end, total phosphorus 930 pounds to the acre	20	0.0093	0.0015	16.1
	40	0.0186	0.0023	12.3
	80	0.0372	0.0036	9.6
	160	0.0744	0.0050	6.7
	320	0.1488	0.0056	3.8
Plot 2. 5-year rotation unlimed end, total phosphorus 844 pounds to the acre	20	0.0084	0.0013	15.4
	40	0.0168	0.0018	10.7
	80	0.0336	0.0025	7.4
	160	0.0672	0.0036	5.3
	320	0.1344	0.0042	3.1
Plot 4. 5-year rotation unfertilized unlimed end, total phosphorus 711 pounds to the acre	25	0.0088	0.00050	5.6
	50	0.0176	0.00084	4.7
	100	0.0352	0.00109	3.0
	200	0.0704	0.00130	1.8

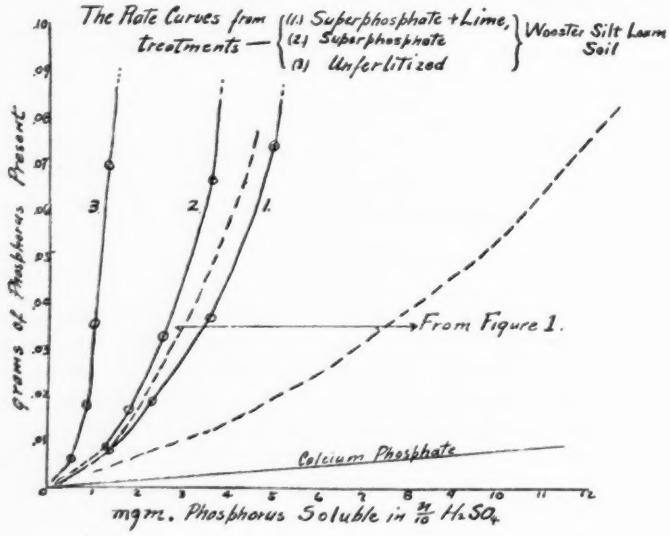
The Rate Curves from treatments — (1) Superphosphate + Lime, (2) Superphosphate (3) Unfertilized
Wooster Silt Loam Soil
Grams of Phosphorus Present
3
2
1
From Figure 1.
Calcium Phosphate
mgm. Phosphorus Soluble in 1/10 H2SO4

FIG. 2

The soluble phosphorus from the unlimed check plot is lower than that from the unlimed phosphated plot. The difference is more clearly indicated by the comparison of the corresponding ratios of soluble to total phosphorus than to the increase in total phosphorus to the acre. The rate curves of solubility in figure 2 show these small differences to be consistent.

The rate curves of the limed end of the phosphated plot 2 appears in figure 2 to the right of the dotted curve for the original soil, and the curve for the unlimed end to the immediate left. With a larger application of superphosphate for the rotation, one would expect more divergence in these two curves. However, the curve for the unlimed end of plot 2 and also the curve for the unlimed check approach the ordinate and agree with those from soils of acid reaction. The ratio of soluble to total phosphorus for this unlimed check plot is lower than for the other plots. According to Lemmermann these soils would respond to additional phosphatic treatment as indicated by their low ratios.

CONCLUSION

The rate curves of two extremes of cropping of a Wooster silt loam soil are shown to diverge from the curves of the original uncropped soil. The greatest change in slope appears for a highly fertilized and limed soil of a 4-year rotation known as the Variety Test Tract. It is evident that the phosphorus is retained in this soil in an effective combination, which has produced high yeilds, and that it remains in a soluble form, which is indicated by the position of the rate curves toward the absissa or approaching the curve for calcium phosphate. The curve for superphosate with lime in a 5-year rotation is found to the right of the curve for the original soil but the divergence is small when compared to the curve of the highly fertilized and manured soil. There is too wide a difference in fertilizer treatment in the two rotations to ascribe the difference to manure entirely.

The curve for the unfertilized continuous corn soil is found beyond the curve of the original soil toward the ordinate, likewise the curve for the unfertilized plot of the 5-year rotation lies nearest the ordinate, or the region of least soluble phosphorus which is combined with iron and aluminum. The study of soils through their rate curves may give more definite information concerning their probable response to phosphatic manuring than a single determination, and it is hoped that this information will become more useful as a greater number of soils of known agricultural history is examined.

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THE MICROFLORA OF LEACHED ALKALI SOILS: II. A LEACHED SODIUM-CHLORIDE SOIL

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There is a wide variation in the toxicity and mode of action of different salts as measured in terms of ammonification, nitrification and nitrogen fixation (2, 5, 6). Some salts are highly toxic to the soil microflora yet do not greatly change the physical and chemical properties of the soils, whereas other salts may greatly change the physical and chemical properties of the soil in addition to being toxic to microörganisms. When tests are made directly in the soil of the bacterial activities, the direct toxic effect of the salt may be masked by the change in the physical and chemical properties of the soil, due to the added salt. In order to eliminate this soil factor, a rather extensive study has been made of the morphology and physiology of typical organisms obtained in pure culture from leached alkali soil. A preceding article contains the description of typical organisms obtained from a leached synthetic alkali soil (1), whereas this paper gives the results of a study of the microörganisms obtained from a natural alkali soil from which the principal soluble salts had been leached. The soil obtained from Corinne, Utah, was high in sodium chloride and devoid of vegetation with the exception of a few highly salt-tolerant plants. Before the present study was made the soil had been leached for 211 days, 79 liters of water passing through it. The principal salts obtained from the 10 kgm. of soil were 379 gm. of sodium chloride and 6 gm. of sodium sulfate.

After the soil was leached, one crop of red clover and two crops of barley were grown on it; it was sampled and tested for bacterial activities after each crop had been removed (3). The leaching greatly increased the bacterial activities and in most cases there was a further increase after the clover and a decrease after the barley. All results indicated an increase in the nitrogen-fixing powers and there were marked gains in soil nitrogen which could be attributed only to microbial fixation (5).

METHOD OF STUDY

After the harvest of the second crop of barley, the soil was carefully sampled and plated on synthetic, Ashby, and nutrient agar in dilutions of 1–20,000

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and 1–200,000. The average for a number of plates was: synthetic agar, 430,000; Ashby agar, 5,350,000; and nutrient agar, 938,000 colonies. Many more colonies developed on the Ashby agar than on either of the other media; moreover, this soil was making a rapid gain in nitrogen. In order that the results may be directly comparable with those reported in the first paper (1) the various types of colonies were fished from Ashby agar plates and obtained in pure culture by repeated plating, after which they were studied and described according to the pure culture methods of the Society of American Bacteriologists.

The ability to bring about beneficial soil changes was determined by the methods given in the first paper (1). The nitrogen-fixing and ammonifying powers are given as the milligrams of nitrogen gained in five weeks by 100 gm. of soil containing 1 per cent mannitol, and the ammonifying powers as the milligrams of ammonia produced in 4 days in 100 cc. of 1 per cent peptone solution.

The organisms were at first cultured on Ashby agar, but after about two months the growth in many cases became sparse and some microörganisms were dying out; consequently, they were all transferred to soil extract Ashby agar, on which they grew well. Thirty-one organisms were obtained and studied in detail, but only a brief description of the more active ammonifiers or nitrogen fixers is given here.

101. Gram-positive, non-spore-forming rods, 0.4 to 0.5 by 0.9 to 1.2μ, occurring singly and in pairs. Motile by means of two polar flagella. Abundant growth on common laboratory media. Liquefies gelatin. Peptonizes milk with an alkaline reaction, produces indol, hydrolyzes starch, produces acid on glucose but not on lactose nor sucrose. Facultative anaerobic. Produces 9.2 mgm. of ammonia from peptone and 17 mgm. from urea, but does not fix nitrogen.

106. Gram-positive, non-spore-forming, non-motile cocci, 0.8 to 1 μ, occurring in clusters. Grows rapidly on agar, slowly on gelatin with liquefaction, moderate orange color growth on potato. Does not produce indol, reduces nitrates, hydrolyzes starch, produces acid on glucose and lactose. Aerobic. Slowly ammonifies peptone and fixes 2.8 mgm. of nitrogen in soil.

107a. A gram-positive, acid-fast capsule, forming rods, 0.7 to 0.8 by 3.2 to 4.8 μ, occurring in chains. Produces central ellipsoidal spores. Rapid growth on all ordinary laboratory media. Rapidly liquefies gelatin and peptonizes milk. Produces indol, reduces nitrates, hydrolyzes starch, produces acid on glucose and sucrose. Facultative anaerobic. Produces 16.9 mgm. of ammonia in peptone, does not fix nitrogen but causes a loss of nitrogen from mannitol containing soil.

108a. Gram-positive motile rods, 0.6 to 0.7 by 3.6 to 4.2 μ. Produces polar ellipsoidal spores. Abundant growth on all ordinary laboratory media, abundant tan growth on potato. Liquefies gelatin and peptonizes milk with an alkaline reaction. Produces indol, does not reduce nitrates, hydrolyzes starch, but does not produce acid on sucrose, lactose, nor glucose. Aerobic. Produces 15.5 mgm. of ammonia in peptone. Does not fix nitrogen.

112c. Gram-positive, spore-forming rods, 0.5 to 0.6 by 2 to 3.8 μ, occurring singly and in clusters. Motile by means of peritrichous flagella. Rapid growth on common laboratory media. Abundant brown growth on potato. Liquefies gelatin, slowly peptonizes litmus milk with the production of alkali and the reduction of the litmus. Does not produce indol, reduces nitrates, and hydrolyzes starch. Aerobic. Produces 25.1 mgm. of ammonia from peptone and 7.7 mgm. from urea, and fixes 1.4 mgm. of nitrogen in soil.

114d. Gram-positive, non-motile cocci, 1.2 by 1.6 μ in diameter, occurring in pairs and clusters. Rapid growth on agar. Slow reddish-brown growth with liquefaction on gelatin. Does not produce indol, reduce nitrates, nor hydrolyze starch. Aerobic. Produces 3.4 mgm. of ammonia in peptone and fixes 5.6 mgm. of nitrogen in soil.

115a. Gram-positive, non-motile cocci, 0.5 to 0.8 μ in diameter, occurring in pairs and clusters. Slow growth on gelatin with liquefaction. Rapid growth on agar and abundant, spreading tan growth on potato. Slowly peptonizes milk with alkaline reaction. Produces indol, does not reduce nitrates, hydrolyzes starch, but does not produce acid on carbohydrate media. Aerobic. Produces 5.4 mgm. of ammonia from peptone and 4.3 mgm. from urea, and fixes 7.0 mgm. of nitrogen in soil.

117. Gram-positive, non-motile, spore-forming, irregular, club-shaped rods, 0.6 to 0.8 by 1.1 to 5.1 μ, occurring singly and in clusters. Abundant growth on agar and potato. Slow growth in broth and gelatin. Liquefies gelatin. Slowly peptonizes milk. Produces indol, does not reduce nitrates, hydrolyzes starch, and does not produce acid on carbohydrates. Produces 5.8 mgm. of ammonia in peptone and fixes 1.4 mgm. of nitrogen in soil.

118b. Gram-positive, non-motile cocci., 0.5 to 0.7 μ in diameter. Liquefies gelatin with the production of a slight yellow pigment. Abundant, smooth, glistening growth on agar. Slimy growth in milk without the production of acid. Produces indol, hydrolyses starches. Produces acid in glucose but not in sucrose nor lactose.

119a. Mold. Mycelium, septate, straight with very little branching. Conidia formed. Grows well on the ordinary laboratory media. Liquefies gelatin. Abundant spreading blue-green growth with yellowing on potato. Slowly peptonizes milk with alkaline reaction. Does not produce indol, reduce nitrates, nor hydrolyze starch. Produces 19.2 mgm. of ammonia in peptone and fixes 2.8 mgm. of nitrogen in soil.

119b. Gram-positive, non-motile, non-spore forming rods, 0.5 to 0.7 by 1.5 to 1.9 μ, occurring in clusters. Abundant growth on agar. Crateriform liquefaction in gelatin. Produces no change in milk, does not produce indol nor reduce nitrates. Hydrolyzes starch, produces acid on glucose but not on lactose nor sucrose. Aerobic. Produces 4.6 mgm. of ammonia in peptone and fixes 4.2 mgm. of nitrogen in soil.

119d. Mold. Mycelium septate with numerous branchings. Conidia formed as in aspergillus. Rapid growth on broth, agar, and gelatin. Liquefies gelatin. Abundant spreading of blue-green growth on potato. Peptonizes milk with alkaline reaction. Produces indol but does not reduce nitrates nor hydrolize starch. Aerobic. Produces 12.4 mgm. of ammonia on peptone but does not fix nitrogen.

119f. Gram-positive, motile, spore-forming rods, 0.6 to 0.7 by 2.4 to 5.1 μ, occurring singly and in clusters. Has peritrichous flagella and produces central spores. Grows rapidly on ordinary media, liquefies gelatin, and peptonizes milk with alkaline reaction. Abundant tan growth on potato. Does not produce indol, reduces nitrates, hydrolyzes starch, and produces acid on glucose but not on lactose nor sucrose; facultative anaerobic. Produces 23.3 mgm. of ammonia on peptone and fixes small quantities of nitrogen in soil.

119g. Gram-positive, non-motile, spore-forming rods, 0.6 to 0.8 by 2.3 to 4.1 μ, occurring singly. Grows rapidly on ordinary media. Brown growth on potato. Rapidly liquefies gelatin and rapidly peptonizes milk with alkaline reaction. Does not produce indol, reduces nitrates, produces acid on dextrose and sucrose, and hydrolyzes starch. Aerobic. Produces 22.9 mgm. of ammonia in peptone.

122a. Gram-positive, non-motile, non-spore-forming rods 1.3 to 1.4 by 4.6 to 13 μ, occurring in chains. Abundant growth on ordinary media. Liquefies gelatin. Slowly peptonizes milk. Produces indol, reduces nitrates, hydrolyzes starch, but does not produce acid on dextrose, sucrose, nor lactose. Aerobic. Produces 4.1 mgm. of ammonia on peptone and slowly fixes nitrogen in soil.

123d. Gram-positive, motile, spore-forming rods 0.7 to 0.8 by 3 to 4 μ. Motile by means of peritrichous flagella. Central ellipsoidal spores. Abundant growth on all common laboratory media. Rapid liquefaction of gelatin. Rapid peptonization of milk. Produces

indol. Reduces nitrates, hydrolyzes starch, produces acid on glucose and sucrose but not on lactose. Facultative anaerobic. Produces 30.1 mgm. of ammonia on peptone but does not fix nitrogen.

TABLE 1

Summary of chief physiological and morphological characters of pure cultures obtained from leached alkali soil

CULTURE NUMBER	NITRATE PRODUCTION	GELATIN LIQUEFACTION	STARCH HYDROLYSIS	MILK PEPTONIZED	INDOL PRODUCTION	LITMUS MILK REACTION	ACTION ON GLUCOSE	ACTION ON LACTOSE	ACTION ON SUCROSE	SHAPE	MOTILITY	GRAM REACTION	CHROMOGENESIS	SPORE FORMATION	AMMONIFICATION—NH_3 PRODUCED	N_2 FIXED IN 100 GM. SOIL
															mgm.	*mgm.*
101	u*	+	−	+	+	−	+	0	0	B	+	+	+	−	9.2	0.0
101b	+	+	+	−	+	+	0	0	0	F	−	+	+	+	3.2	0.0
105e	−	+	+	+	0	+	u	0	+	F	−	+	−	+	4.3	0.0
106	+	+	+	−	−	+	+	0	0	C	−	+	+	−	1.5	2.8
107a	+	+	+	+	+	0	+	0	+	B	+	+	−	+	16.7	−2.8
108a	−	+	+	+	−	−	0	0	0	B	+	+	−	+	15.5	0.0
108b	−	+	+	+	+	−	0	0	0	C	−	+	−	−	4.9	0.0
111a	−	−	−	−	−	0	+	0	0	C	−	+	+	−	1.9	2.8
112a	−	−	+	−	−	+	0	+	0	C	−	+	+	−	2.2	0.0
112c	+	+	+	+	−	−	+	0	0	B	+	+	+	+	25.1	1.4
113a	+	+	+	−	+	+	0	+	0	B	−	+	−	−	0.0	u
113b	−	+	−	−	−	+	0	0	+	B	−	+	+	+	0.0	u
114d	−	+	−	−	−	+	0	0	0	C	−	+	−	−	3.4	5.6
115a	−	+	−	+	+	−	0	0	0	C	−	+	−	−	5.4	7.0
115b	−	+	+	+	−	−	+	0	0	C	−	+	−	−	4.4	0.0
115c	−	+	+	−	−	+	0	0	0	C	−	+	−	−	u	u
116	−	+	+	+	+	−	0	0	0	C	−	+	−	−	1.4	0.0
117	−	+	+	+	+	0	0	0	0	B	−	+	−	−	5.8	1.4
118b	u	+	+	−	+	0	+	0	0	C	−	+	−	−	1.5	1.4
119a	−	+	+	+	−	−	0	0	0	F	−	+	+	+	19.2	2.8
119b	+	+	+	−	−	0	+	0	0	B	−	+	−	−	4.6	4.2
119d	−	+	−	+	+	−	0	0	0	F	−	+	+	+	12.4	0.0
119f	+	+	+	+	−	−	+	0	0	B	+	+	−	+	23.3	1.4
119g	+	+	+	+	−	−	+	0	+	B	−	+	+	+	22.9	0.0
122a	+	+	+	+	+	−	0	0	0	B	−	+	+	−	4.1	1.4
122b	+	+	+	−	+	−	u	0	0	B	−	+	−	−	2.4	0.0
123b	+	+	+	−	+	+	+	+	0	B	−	+	−	−	2.7	0.0
123c	+	−	+	−	−	+	0	0	0	B	−	+	−	+	6.0	0.0
123d	+	+	+	+	+	0	+	0	+	B	+	+	−	+	30.1	0.0
125c	+	+	+	+	+	+	+	0	+	B	+	+	−	+	37.7	0.0
125e	−	+	+	+	+	−	u	0	0	F	−	+	+	+	2.9	0.0

* u = undetermined.

125c. Gram-positive, motile, spore-forming rods 1.2 to 1.3 by 3.2 to 4 μ, occurring in chains. Motile by means of peritrichous flagella. Rapid growth on ordinary laboratory media. Abundant light tan growth on potato. Liquefies gelatin, rapidly peptonizes litmus milk with the reduction of the litmus. Reduces nitrates, hydrolyzes starch, decomposes

cellulose, and produces acid on glucose and sucrose but not on lactose. Facultative anaerobic. Produces 37.7 mgm. of ammonia in peptone, does fix nitrogen.

A summary of the chief physiological and morphological properties of the 31 organisms obtained in pure culture and studied in detail is given in table 1.

Of the 31 gram-positive microörganisms isolated from a leached sodium chloride, soil, 16 were bacilli, 10 cocci, and 5 filamentous. Twelve of them were chromogenic under one or more conditions studied. All but 3 of the organisms liquefied gelatin, 25 hydrolyzed starch, 18 peptonized milk, 11 fermented glucose—6 of these fermented sucrose and 3 fermented lactose—and 90 per cent of the organisms decomposed peptone with the production of ammonia. The quantity produced in 4 days in 100 cc. of a 1 per cent peptone solution varied from 1.4 mgm. to 37.7 mgm. Six of the organisms decomposed urea with the production of ammonia, the quantity varying from 3.4 to 10.2 mgm. One of the organisms decomposed cellulose and 11 caused gains of nitrogen when incubated in soil containing mannitol, 2 showed gains of over 5 mgm. of nitrogen in 100 gm. of soil in five weeks. None could be shown to fix nitrogen in pure nitrogen-free synthetic media.

SUMMARY

A study was made of the organisms developing on Ashby agar and obtained from a natural-occurring, sodium-chloride-alkali soil which had been leached until most of the soluble chlorides had been removed. Thirty-one organisms were obtained in pure culture. Of this number 11 fixed nitrogen. The gains vary from 1.4 to 7 mgm. to 100 gm. of soil. Thirteen of the organisms produced over 5 mgm. of ammonia in 4 days when grown in a 1 per cent peptone solution. All but two produced ammonia. Only 1 of these orgaisms was able to decompose cellulose, whereas 6 decomposed urea. The nitrogen-fixing ability of the organisms varied from 1.4 to 7 mgm. Three of the total number fixed over 4 mgm. in the 5-week incubation period when inoculated into sterile soil which contained 2.5 gm. of mannitol.

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IMPRESSIONS OF CERTAIN SOIL CONFERENCES IN EUROPE, DURING THE SUMMER OF 1929, AND SOME PLANS FOR THE SECOND INTERNATIONAL CONGRESS OF SOIL SCIENCE IN 1930

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Preparatory to the Second Congress of the International Society of Soil Science, which is scheduled to take place in Russia during the summer of 1930, the various international commissions and sub-commissions met this summer, between May 20 and July 27, in various European cities to discuss their problems and to organize the program for the Russian congress. The first commission met in Prague, the second and the alkali sub-commission in Budapest, the third and the forestry sub-commission in Stockholm, the fourth in Königsberg, the fifth in Danzig, and the sixth in Prague. In addition to transacting official business, which in some instances played only a secondary rôle at the meetings and in the reports of various committees, each commission arranged a scientific program. Usually from two to ten sessions, depending upon the commission and the number of members attending the meetings, were devoted to the reading of papers and to scientific discussions. In some cases, the meetings resembled that of an international congress rather than of a single commission conference. Some commissions arranged various excursions for soil examinations in the region where the conference took place; some of these excursions, like the one following the Budapest meeting, lasted for nearly a week. The field excursions were very well organized and served the further purpose of bringing about a better understanding of the nature of the soil as formed under the various climatic conditions, and of the problems underlying its investigation and utilization.

The meetings were distinctly international in nature, since they were attended by representatives from ten to twenty different countries. For instance, at the meetings of the second commission on soil chemistry, nearly one hundred scientific workers were gathered, sixty of whom came from twenty or more different countries outside of Hungary. There were only three American representatives attending the meetings of some of the commissions, namely that of the second, third, fourth, and fifth. The meetings of the first and sixth commissions were not attended by any American delegate. However, the International Congress of Forestry Experimental Stations which arranged, in cöoperation with the meetings of the forestry sub-commission, a rather extensive and important program on forest soils, was attended by more than a dozen American delegates.

The author attended the meetings of the second and third commissions, as

well as of the subcommissions (of the fifth commission) on alkali and forest soils. He also took part in the Congress of Forestry Experimental Stations. He feels, therefore, justified in coming to certain general conclusions concerning the status of soil investigation, as presented at these conferences.

The first general impression from these conferences and congresses is the international spirit guiding the development of the young but active science of the soil. From a handful of people, primarily interested in soil classification, that met first in Budapest in April, 1909, and organized the Agrogeological Commission, there grew, largely after 1922, a large organization with a membership of over one thousand in all civilized countries of the world. This society, the International Society of Soil Science, is divided into six well-organized commissions with about as many sub-commissions. These cover all phases of soil science and its relation to plant growth and crop production. The society also has twenty well-organized national sections which work towards the advance of the international idea of soil science as an independent science. When one compares the Prague conference in 1922 and the Rome conference in 1924 with the first congress of the recently organized society, held two and a half years ago in Washington, and with the plans for the second congress in Russia during next year, one marks a rapid increase in membership, a growing interest in the subject, and a steady development of the various phases of soil science embraced by the society. The interest aroused by these conferences in the government circles and in the populace, as shown by the participation of government representatives at the social functions and by the attention devoted by the press, is evidence of the growing recognition that the subject is receiving. The time is not far distant when the science of the soil will be recognized, as it has been so far only in Russia, as an independent science, just as is geology, botany, or zoölogy, and not merely as a branch of agriculture. This depends largely, of course, upon the soil scientists themselves.

As to the programs of the international gatherings, the following general tendency was apparent: Instead of inviting the presentation of numerous papers dealing with all phases of soil science, frequently outside of the boundaries of the particular commission, several of the commissions devoted their meetings to a discussion of only a limited number of subjects, previously decided upon. The contributions to the second international congress are likewise to be limited to particular subjects. In some cases, a part of the program will actually be limited to invitation papers.

The third commission, for example, decided to devote their meetings during the congress to a discussion of only three subjects: 1. The problem of fixation of nitrogen by microörganisms, both symbiotic and non-symbiotic. Three entire sessions will probably be devoted to a discussion of the present status of this most important problem. 2. The decomposition of organic matter by microörganisms, and its application to agricultural practice. 3. Methods for studying microbiological processes in soil. At a special request of the Russian

group, representing this commission at the second congress, probably one session will be devoted to the subject of the distribution of microörganisms in soil profiles. If one studies the program of this commission during the Rome and Washington congresses and the plans for the Russian congress, one finds a gradual narrowing of the number of subjects submitted for discussion and a broadening of the attention paid to each particular subject.

Similar observations could be made in regard to the second commission on soil chemistry. Only four subjects were brought out for discussion at the Budapest meeting: 1. The hydrochloric extract of soil. 2. The nature of soil acidity. 3. The determination of nutritive elements in the soil. 4. The nature of soil organic matter. The second commission has four standing committees, which are to report in detail on the status of these subjects at the various conferences and to assist the president of the commission in the preparation of the scientific program. It may be of interest to note that all the proceedings of the second commission and of the alkali sub-commission were published previous to the meetings in two volumes and were available to the delegates at the opening of the conference.

At the joint meetings of the forestry sub-commission and the soil section of the forestry congress, the "organic matter" question occupied a prominent place. This included discussions of the nature of the soil organic matter or "forest soil humus," together with the decomposition processes, especially the liberation of nitrogen in an available form (as ammonia, nitrate), and of CO_2. The organic matter program was followed by a separate program and discussion on soil types and natural forest types, problem of water-relations, and treatment of podsol soils.

The fifth commission was devoted largely to a discussion of the problem of "Braunerde," to determining the principles underlying the various characteristics of podsols and chernozems, and to the choice of a system of coloring and marking for the general soil map of Europe. It is interesting to note that the summary report of this conference is concluded as follows: "So the congress has attained its purpose—to call forth new ideas, to show new ways for international collaboration in solving the problems of the soil." The preliminary program of work of the fifth commission at the congress in Russia includes the following subjects for discussion: 1. The genesis of soil; 2. Classification and nomenclature of soils; 3. Geography and cartography of soils.

The sixth commission decided to bring up for discussion at the Russian congress the following three subjects: 1. Problems of drainage; 2. Water relations in soil; 3. Field irrigation. The session of the sub-commission for peat soils will be devoted to the reading and discussion of papers dealing with: 1. Stratigraphy; 2. Profile analyses; 3. Cultural operations of peatlands. A comparative study of certain regional areas of low moor also has been proposed, with the view to adopting a uniform procedure and terminology in peat investigations.

FIG. 1. EXCURSIONS OF THE SECOND INTERNATIONAL CONGRESS OF SOIL SCIENCE, 1930

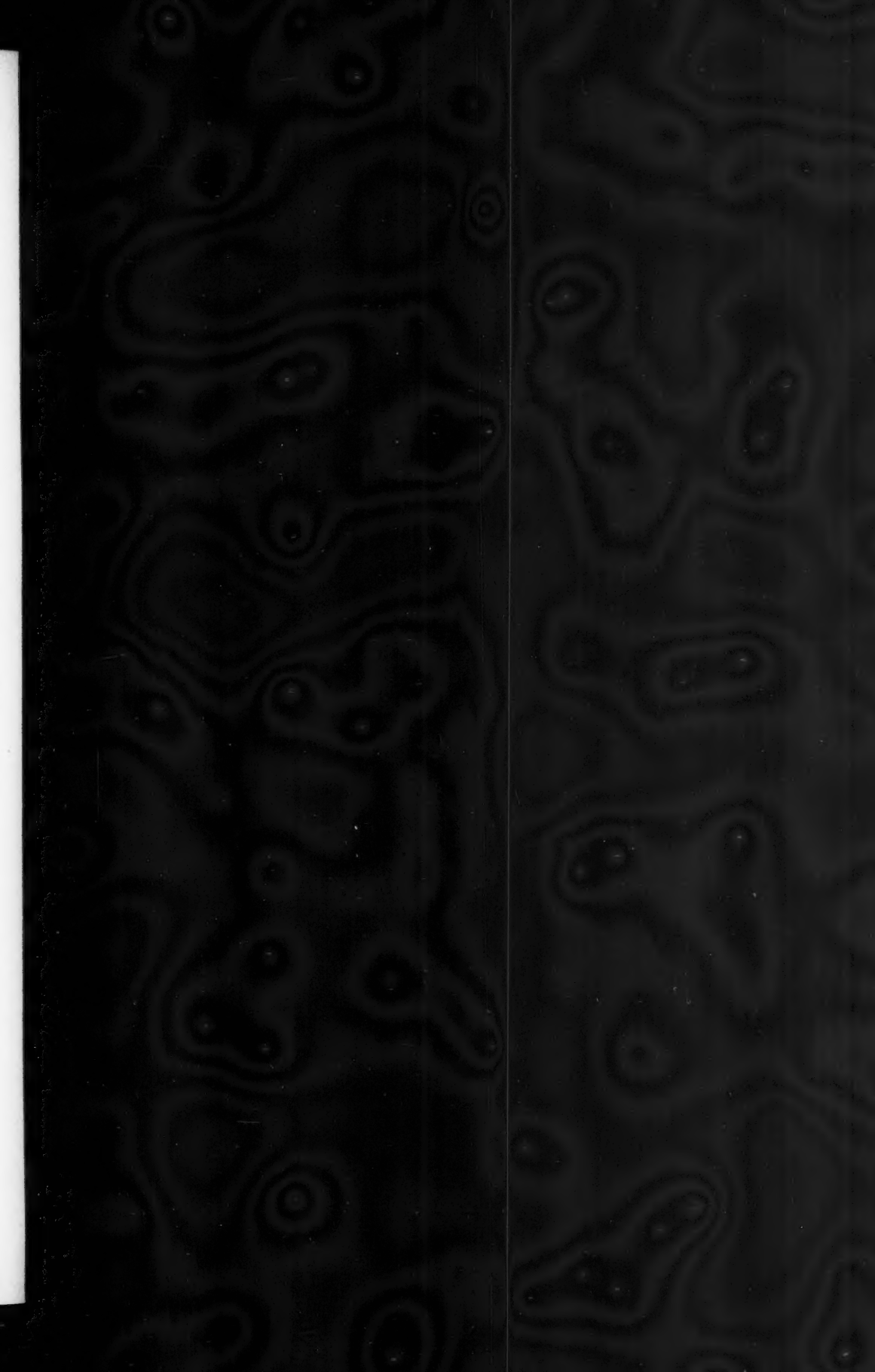
It is of interest to consider the languages used at these international gatherings. Only a decade or two ago, French predominated at the various international conferences, with German frequently taking first place, especially during the scientific discussions. During the conferences that met this year, French was almost nonexistent or was reduced to a poor third place, not merely because French scientists were absent or were few in number, but also because the Russian, Polish, and other scientists who previously used the French language only, have learned to speak German and English. It is interesting to note the progress of the English language; it is a close second to German in the number of papers presented in that language and during the discussion. Many Danish, Swedish, Norwegian, as well as Eastern and Central European scientists prefer, or are able to use, this language. German, however, predominated, especially in view of the fact that most of the conferences took place in Germany itself or in countries formerly forming a part of Austria-Hungary.

As to the plans for the Second International Congress, the writer visited Moscow this summer and learned the plans of the Russian organizing committee at first hand. The congress will begin in Leningrad on July 20, 1930 and will last there six days, two of which will be devoted to excursions. All the delegates will then proceed to Moscow, where the congress will be continued for another six days, including two more excursion days. In all, eight days will be devoted to scientific conferences and discussions, eight sessions to the meetings of the society as a whole (plenary) and the other eight sessions to commission conferences. Soil exhibits will be held both in Leningrad and in Moscow, the more scientific in the former city and the more applied in the latter city.

At the completion of the congress, the delegates will leave, on special trains, for a 29-day excursion through central and southern Russia crossing all the soil zones of the European part of U. S. S. R., as shown in figure 1. This ought to give the European and American delegates an opportunity to familiarize themselves with the various soil types made classical by the investigations of the Russian School of pedologists, who have given to soil science such brilliant names as that of Dokutchaiev and Sibirtzev. During the trip, members of the congress will have an opportunity to visit agricultural experiment stations and large agricultural and industrial enterprises. Three of the 29 days will be allowed for rest, two in the Caucasus and one in the Ukraine.

If desired, special excursions may be arranged to the Mourman peninsula for visiting peat soils, forest swamps, and the biological station; to Central Asia; or to the agricultural exhibit of Minsk (White Russia).

Information about costs can be obtained from Dr. A. G. McCall, United States Department of Agriculture, Washington, D. C.

The organizing committee has announced that all persons desiring to attend the congress are requested to advise Prof. Dr. A. Yarilov, President of the organizing committee, Moscow, Karuninskaja, 1, Gosplan before February 1,

1930. The committee also requests that summaries of all reports (supplied with figures and graphic tables) intended for the congress be written in English, German, or French (if possible in all three languages) and sent at once to the presidents of the respective international commissions of the International Society of Soil Science, either directly or through the organizing committee. The complete papers, in any of the three languages, should be brought to the congress to be left there for printing.